THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, October 3rd, 1956, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. K. A. Williams, A.Inst.P., M.Inst.Pet., F.R.I.C.

The subject of the meeting was "Chromatography" and the following papers were presented and discussed: "The Determination of Vitamin D and Related Compounds. Part I: Introduction and Preparation of Compounds in the Irradiation Series. Part II: Analysis of Irradiation Products," by W. H. C. Shaw, F.P.S., F.R.I.C., J. P. Jefferies, B.Sc., A.R.I.C., and T. E. Holt, B.Sc., A.R.I.C.; "Some Examples of the Use of Paper Chromatography in Toxicological Analysis," by A. S. Curry, M.A., Ph.D.

DEATHS

We record with regret the deaths of

Mervyn Henry Jenkins
William Henry Jackson.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, September 11th, 1956, in the Mason Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Chairman of the Section, Mr. J. R. Leech, J.P.

The following paper was presented and discussed: "Recent Advances in the Analysis of Cast Iron and Foundry Materials," by W. E. Clarke, A.R.I.C.

The Microbiological Assay of Penicillin in Feeding Stuffs

By J. S. SIMPSON and K. A. LEES

(*Presented at the meeting of the Biological Methods Group on Friday, December 9th*, 1955)

A microbiological plate method, with *Sarcina lutea* as test organism, is described for the assay of penicillin in feeding stuffs. The method is capable of determining penicillin in samples containing less than 5 units per g, at which level plate assay methods with *Bacillus subtilis* are insufficiently sensitive.

Samples are extracted with 25 per cent. v/v acetone buffer solution and diluted to levels of 0·1 and 0·05 units per ml. The solutions are employed directly in a standard cup-plate microbiological assay. Standard reference solutions of penicillin are also prepared in the solvent solution.

The limits of error ($P = 0{\cdot}95$) of the method are of the order of 90 to 112 per cent. with six samples and two standards at two levels each on an 8×8 quasi Latin-square plate.

In the absence of any physical and chemical methods for estimating low antibiotic levels in crude materials, the microbiological assay of procaine penicillin in animal feeding stuffs has assumed considerable importance. The penicillin concentration in feeding stuffs may range from 60 units per g to as low as 0·1 units per g. For the lower levels it is necessary to have a sufficiently sensitive method of assay that retains a standard error of not more than about $\pm$ 10 per cent.

Published work on the assay of penicillin in feeding stuffs is limited. Esposito and Williams[1] employ a method in which methanolic extracts are placed by means of a pipette on to paper discs laid on the surface of assay plates seeded with *Staphylococcus aureus*. This method was later modified by incorporating succinylsulphathiazole in the assay medium to obtain increased sensitivity.[2]

Price and Boucher[3] describe a similar method in which *Bacillus subtilis* was the test organism.

Trials of these two methods[2,3] in our laboratories showed that they were somewhat lengthy and laborious and, moreover, were not sufficiently sensitive for low-potency samples.

The large-plate method described in this paper was developed to be free from these defects while permitting a high throughput of samples with a standard error of about $\pm$10 per cent.

Experimental

A large-plate microbiological assay of penicillin in animal sera, based on the Food and Drugs Administration method, has been developed and used in these laboratories for some time: in it, *Sarcina lutea* is used as test organism to determine penicillin serum concentrations down to about 0·03 units per ml. Preliminary experiments confirmed that an adaptation of this method could be used for the assay of penicillin in compound feeding stuffs.

Test organism—

Sarcina lutea (N.C.I.B. 8553) was the test organism selected, its use being described originally by Randall.[4] Freeze-dried cultures are reconstituted in our laboratories at 3-monthly intervals; from "smooth" colonies exhibiting chromogenesis, subcultures are inoculated on slopes of the assay medium and incubated at 30° C for 24 hours. Bacterial suspensions are then prepared by washing the growth from the slope with sterile saline and adjusting to match a Wellcome opacity tube No. 7. Of this suspension 0·6 ml is added to 70 ml of the melted assay medium previously cooled to 47° C. Routine checks are made by microscopical examination of Gram-stained films from each subculture and suspension. We have found *Sarcina lutea* to be an organism prone to variation arising from inconstant subculturing routines; adherence to a proved successful subculturing procedure is of prime importance.

ASSAY MEDIUM—

The assay medium had the following composition—

Lab Lemco (Oxoid)	1·5 g
Yeast extract (Bacto)	3·0 g
Peptone (Bacto)	6·0 g
Dextrose (analytical-reagent grade) ..	1·0 g
Agar (Davis)	12·5 g
Distilled water to	1 litre

PREPARATION OF ASSAY PLATES—

Pour 180 ml of melted medium into levelled 12-inch × 12-inch square plates, previously sterilised. When this medium has set, pour in 70 ml of the inoculated medium so as to form a two-layer medium, allow this to set, and transfer the plates to a refrigerator until required for punching out the assay cups. Because of the extreme sensitivity of the test organism to penicillin, it is important that the exposure of the plates to the atmosphere be reduced to a minimum: if possible the assays should be carried out in a separate room where interference from dust containing antibiotic material is negligible.

TABLE I

EFFECT OF ACETONE ON PENICILLIN RECOVERY BY MICROBIOLOGICAL ASSAY: TEST ORGANISM, *Sarcina lutea*

	Acetone in extracting medium, % v/v	Recovery, %
Baby chick mash	nil	56
	12·5	88
	25	96
Sow and weaner meal ..	nil	44
	12·5	64
	25	92
Pig meal	nil	54
	12·5	73
	25	98

EXTRACTION OF SAMPLES FOR ASSAY—

Extraction of penicillin from feeding stuffs by aqueous phosphate buffer solutions leads to poor recovery of penicillin. The most satisfactory solvent for good recovery has been found to be 25 per cent. v/v of acetone in pH 7·5 phosphate buffer solution (Table I). Other solvents have been used, but we have found methanol and ethanol to interfere with the growth of *S. lutea*. Solutions containing higher concentrations than 25 per cent. v/v of acetone have presented problems, especially during the plating-out procedure, and experiments have shown no improved recovery of penicillin at greater concentrations of acetone than 25 per cent. The recommended procedure is to shake 20 g of the sample for 1 hour at room temperature with a volume of solvent depending on the expected potency of the material. Thus, for example, 20 g of a feeding stuff containing approximately 1 unit of penicillin per g are shaken with 50 ml of solvent. The suspension is then allowed to settle for a few minutes and 10 ml of the supernatant solvent solution are taken to prepare further solutions containing 0·1 and 0·05 units per ml in the solvent. Whenever possible this procedure is conducted in duplicate. In order to maintain expeditious daily working, we have felt justified in not resorting to percolation methods or other techniques involving filtration and subsequent washing of the bed.

PREPARATION OF STANDARD SOLUTIONS—

As standard, we use a crystalline preparation of sodium benzylpenicillin of low moisture content that has been standardised against the British National Standard. For the assay of feeding-stuff extracts in 25 per cent. acetone - phosphate buffer, standard solutions are prepared in an identical solvent, since the results from either *B. subtilis* or *S. lutea* are depressed by the presence of 25 per cent. of acetone in the sample dilutions if no compensating solvent is present in the reference standard dilutions. The degree of depression was about 25 per cent. for *B. subtilis* and 27 per cent. for *S. lutea* (see Table II). The primary standard

solutions are prepared twice weekly and diluted daily to plating strength. The freshly prepared dilute solutions are checked by assay on the day of preparation, for use on the following day if satisfactory.

TABLE II

EFFECT OF ACETONE ON STANDARD PENICILLIN SOLUTIONS

All solutions contained sodium benzylpenicillin (0·1 unit per ml) and were assayed against aqueous standard buffer. Test organism, *Sarcina lutea*

Acetone in test solution, % v/v	Apparent potency (standard buffer taken in 100), %
nil	100
12·5	80
25	73·5 (mean of 15)
50	53

ASSAY DESIGN—

The usual (2 + 2) assay, with 8 × 8 quasi Latin-square designs, as described by Lees and Tootill,[5,6,7] has been employed for most samples. All samples have been assayed by two assistants using separate dilutions, plates and designs. The zone diameters, after incubation of plates at 30° C for 18 to 24 hours, are measured with needle-point calipers (by independent assistants) directly on the surface of the medium. All assay plates accommodate six samples each at two levels and two standard solutions, one of which is the reference standard for the day, prepared on the previous day and found satisfactory by assay. The zones of inhibition with *S. lutea* are generally well defined, but not invariably so, although they are with *B. subtilis*.

RESULTS

DOSE - RESPONSE RELATIONSHIP—

Fig. 1 shows the effect of various concentrations of penicillin against *S. lutea* on the diameters of the zones of inhibition.

A 6 × 6 Latin-square design of the large-plate assay of penicillin in feeding stuffs is illustrated in Fig. 2; this accommodates two samples and one standard at two levels each.

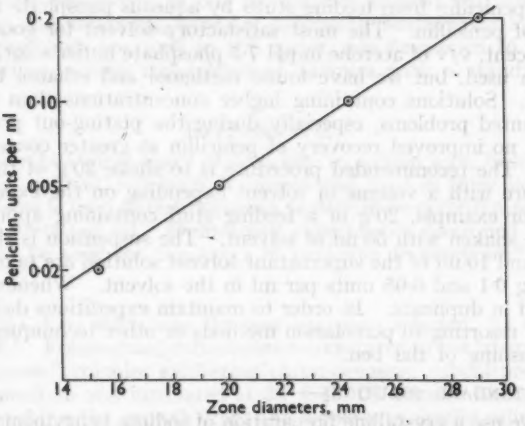

Fig. 1. Microbiological assay of penicillin in compound feeding stuffs, with *Sarcina lutea* as test organism. Each point is the mean of four observations

The results obtained from one batch of feeding stuffs by three operators on five successive days are shown in Table III.

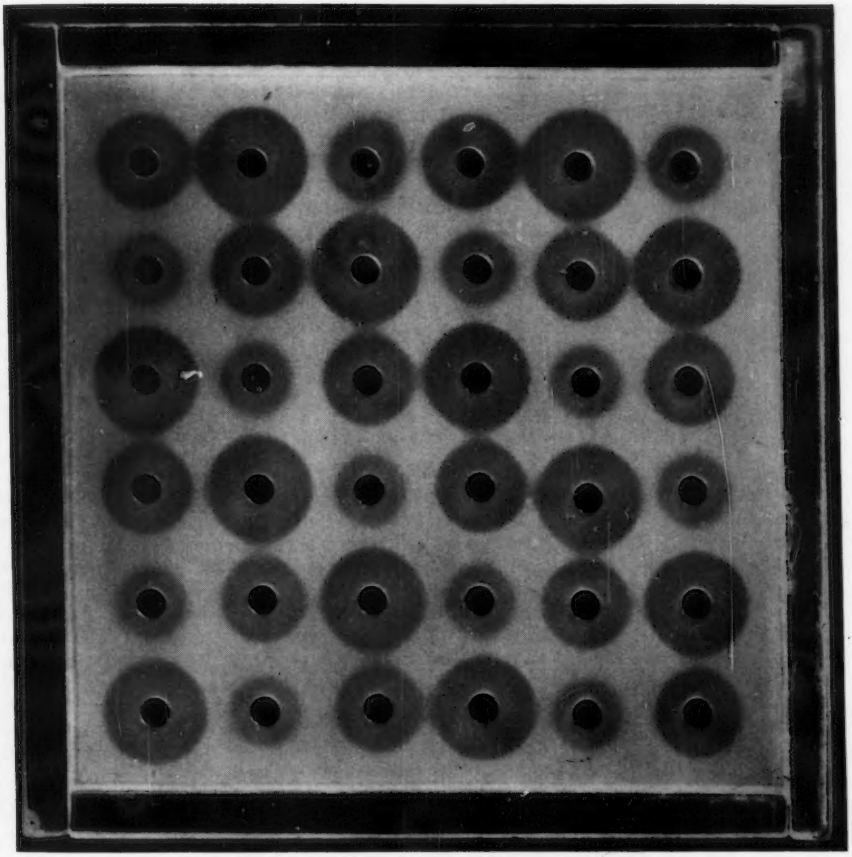

Fig. 2. A 6 × 6 Latin-square design in the large-plate assay of penicillin in feeding stuffs, with *Sarcina lutea* as test organism, accommodating two samples and one standard at two levels each

[To face p.564

TABLE III

MICROBIOLOGICAL ASSAY OF SODIUM BENZYLPENICILLIN: TEST ORGANISM, *Sarcina lutea*

Day	Operator 1	2	3	Mean
1	4·16	6·22	3·00	4·46
2	3·76	3·95	3·84	3·85
3	3·62	3·78	4·05	3·82
4	3·37	3·41	4·36	3·71
5	5·64	3·78	3·62	4·35

Mean of all assays = 4·04 units per g.

Several individual assays have been done by different operators with different suspensions from different samples of the various feeding stuffs and the potency estimates from these assays were statistically analysed. The analyses showed no significant differences between operators, suspensions or various samples of each feeding stuff. In view of the consistency of these assays the standard deviation of each assay was calculated from the replicate potency estimates. These results are summarised in Table IV.

TABLE IV

STANDARD DEVIATION OF AN ASSAY

Feeding stuff	Mean assay of experiment	Percentage standard error of an essay
Sow and weaner meal	3·05	3·82
Pig meal (Amvilac No. 1)	25·25	5·57
Pig meal (Amvilac No. 2)	8·04	8·57
Turkey starter pellets	5·02	5·27

DISCUSSION OF RESULTS

Esposito and Williams[1] have emphasised the need for using a modified standard with a methanolic extract of the unfortified feeding stuff as diluent. This observation was confirmed by Price and Boucher,[3] but from the point of view of routine working such a procedure has a serious disadvantage, since it is necessary to use a separately prepared reference standard for each type of sample. The use of *Sarcina lutea* as the test organism, for which samples require diluting to the low levels of 0·1 and 0·05 units per ml, avoids the need to employ control extracts of unfortified feeding stuffs, the use of a standard prepared in the solvent solution only (25 per cent. v/v acetone - buffer) being adequate. This in turn enables more samples to be assayed on each plate. The tedium of repeated applications of the test solutions to paper discs is also avoided, since the routine cup - plate procedure gives satisfactory results.

Should further increased sensitivity be required, we suggest that the medium described by Brown and Binkley,[8] who examined the growth requirements of *S. lutea*, may be studied, for we have, on occasion, been able with it to use reference standards of as little as 0·01 and 0·005 units of penicillin per ml.

Our thanks are due to Mr. L. J. Hamilton for much helpful advice, to Mr. J. P. R. Tootill for the statistical analyses of results and to Mr. W. H. C. Shaw and Mr. J. P. Jefferies, who collaborated in some of the work involved in extracting the feeding stuffs.

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May 9th, 1956

An Absorptiometric Determination of Iridium

By S. S. BERMAN and W. A. E. McBRYDE

A reaction between stannous chloride and iridium salts in a medium of hydrobromic acid produces an intense yellow colour suitable for an absorptiometric determination of iridium. The method is more sensitive than any published so far. Several factors influencing the intensity and stability of the colour are described. The nature of the coloured species is unknown. Iridium may be determined in samples that have been heated to fumes with sulphuric acid. Owing to interference from the other platinum metals, it is necessary to isolate the iridium before this determination is made.

Of absorptiometric procedures that have been investigated for the determination of iridium, none has proven satisfactory for certain types of samples, because of insufficient sensitivity, lack of precision or interference by sulphuric acid. Concerning sensitivity, in Table I are given the published sensitivities of several methods for iridium expressed (*i*) as the molar extinction coefficient, and (*ii*) as the concentration required to produce an optical density of unity in a 1-cm cell. Some of these methods cannot be applied to solutions that have been fumed with sulphuric acid (as for the destruction of organic matter).

Table I

Sensitivity of absorptiometric methods for iridium

Method	Molar extinction coefficient	Concentration for an optical density of 1·0
Perchloric acid - phosphoric acid[1]	$4{\cdot}57 \times 10^3$	42·2
Sulphuric acid - ceric sulphate[2]	$1{\cdot}79 \times 10^3$	108
p-Nitrosodimethylaniline[3]	$1{\cdot}93 \times 10^4$	10·0
Ethylenediaminetetra-acetic acid[4]	$3{\cdot}2 \times 10^3$	60
o-Dianisidine[5]	$1{\cdot}25 \times 10^4$	15·5
Stannous chloride - hydrobromic acid ..	$4{\cdot}96 \times 10^4$	3·9

Although stannous chloride forms a coloured product when heated with iridium chloride, this reaction cannot apparently be made the basis of an absorptiometric method for iridium.[6,7] We have discovered that, when the stannous chloride and iridium solutions contain appreciable concentrations of hydrobromic acid, a sensitive colorimetric procedure is possible. Our experiments do not permit us to speculate upon the identity of the coloured product, but evidence from the analogous reaction with platinum may be adduced to suggest its probable complexity.[8] In view of the familiar colour-forming reactions of the other platinum metals

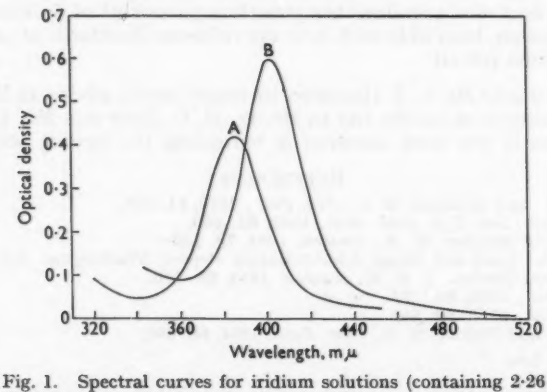

Fig. 1. Spectral curves for iridium solutions (containing 2·26 p.p.m. of iridium): curve A, colour developed with stannous chloride in hydrochloric acid; curve B, colour developed with stannous chloride in hydrobromic acid

when treated with stannous chloride, the use of this procedure is necessarily restricted to determinations in which the iridium has been first isolated.

We first noted that chloro-iridate solutions that had been boiled with hydrobromic acid produced a bright yellow solution upon the addition of stannous chloride solution in hydrochloric acid. This colour is quite stable and produces a spectral curve (Fig. 1) with an absorption peak at 385 mμ. The formation of this colour, however, was found to be quite susceptible to interferences. If stannous chloride dissolved in hydrobromic acid is used as the colour-forming reagent, a stable yellow solution is obtained, producing a spectral curve with an absorption peak at 402 mμ (Fig. 1). This coloured solution was found to be even more stable than the former, and could tolerate the presence of diverse ions to a much greater degree.

Experimental

Apparatus and solutions—

Absorption measurements were made with a Beckman Model DU quartz spectrophotometer and matched 1·00-cm Corex-glass cells.

A standard iridium solution was prepared by dissolving very pure ammonium chloroiridate in 0·1 *M* hydrochloric acid. This solution was standardised by potentiometric titration with ferrous sulphate,[9] and found to contain 1·89 mg of iridium per ml of solution. A 5-ml aliquot of this solution was diluted to 500 ml with 0·1 *N* hydrochloric acid, and so contained 18·9 μg of iridium per ml of solution.

A rhodium solution was prepared from pure sodium chlororhodite, and solutions of the other platinum metals from weighed portions of the pure metallic sponge.

The reagent was a 25 per cent. w/v solution of stannous chloride dihydrate dissolved in concentrated hydrobromic acid (about 40 per cent. of HBr). The hydrobromic acid was redistilled before use to remove any impurities, chiefly bromine.

All other chemicals used were of analytical-reagent or chemically pure grade.

Development of the method—

Rate of colour development—The colour does not develop at room temperature. The development is immediate in a boiling solution of iridium in hydrochloric acid, but since the time of heating the solution must be rather closely controlled (see below) and since the solutions tended to bump, the use of boiling solutions was found to be inconvenient.

Colour development was found to be complete within 1 minute (except for samples that had been heated with sulphuric acid, when 2 minutes were required) at the temperature of a boiling-water bath.

Effect of heating time—If the solutions are kept in the water bath for more than 2 minutes after the reagent is added, the optical density decreases, the decrease being proportional to the time of heating. The optical density decreases even if the solutions are removed from the water bath and allowed to cool slowly at room temperature. For this reason solutions must be quickly cooled under a stream of cold water on removal from the water bath.

Quantity of reagent added—The quantity of reagent used does not affect the optical density of the solution at 402 mμ (measured against a reagent blank). However, the stability of the coloured species is increased the greater the quantity of reagent used. With 1 ml of reagent the intensity of the colour begins to fade within 15 minutes. With 3 ml of reagent the colour is stable for about 3 hours and with 5 ml the colour does not fade in 6 hours.

Quantity of hydrobromic acid—Up to 8 ml of hydrobromic acid added to the sample do not affect the optical density in this procedure. With more than this quantity of acid present the absorption decreases. The presence of free bromine in the hydrobromic acid was found to decrease the intensity of the colour.

Reaction with stannous bromide—The effect of using stannous bromide in place of stannous chloride was examined. The stannous bromide was prepared in two ways. Tin foil was dissolved in hydrobromic acid in one procedure, and stannic bromide was dissolved in hydrobromic acid and reduced to stannous bromide by metallic tin in the other. With both solutions the same spectral curve with a peak at 402 mμ was obtained, but the molar extinction coefficient was about 10 per cent. greater than that obtained when stannous chloride was used. This gain in sensitivity was offset by the fact that the coloured species was less stable and faded rapidly.

RECOMMENDED PROCEDURE—

Transfer the iridium solution to a 20-ml test-tube and dilute to 5 ml with water. Add 5 ml of concentrated hydrobromic acid and place the tube in a boiling-water bath. After 10 minutes add 5 ml of the stannous chloride reagent. Remove the test-tube from the water bath exactly 2 minutes after the addition of the reagent, and immediately cool the tube and contents under a stream of cold water. Transfer the solution to a 25-ml calibrated flask and dilute to volume with water. Measure the optical density of the solution at 402 mμ, using a reagent blank for comparison.

PRECISION ATTAINABLE—

The mean optical density of eight replicate samples each containing 56·7 μg of iridium in a final volume of 25 ml was 0·596. The mean deviation of these results was 0·001(5) or 0·25 per cent.

ADHERENCE TO BEER'S LAW—

The coloured solutions adhere to Beer's law at concentrations up to 3 p.p.m., above which there is a slight falling off in optical density with increased concentrations; this is shown by the following results—

Concentration (c), μg per ml ..	0·756	1·51	2·27	3·02	3·78
Optical density (d)	0·201	0·396	0·597	0·795	0·971
Ratio d/c	0·266	0·262	0·263	0·263	0·257

The recommended range for this method is from 0·5 to 3·0 p.p.m.

DETERMINATION OF IRIDIUM IN SOLUTIONS AFTER FUMING WITH SULPHURIC ACID—

This colorimetric procedure arose from our need of a sensitive method for the determination of iridium in solutions that had been fumed with sulphuric acid. This treatment converts rhodium and iridium to sulphate complexes, which frequently fail to give reactions characteristic of the same metals in chloride solutions. The most sensitive absorptiometric procedure for iridium heretofore published is not applicable in the presence of non-volatile acids.[3]

It was found that up to 2 ml of concentrated sulphuric acid in the iridium solution (not fumed) produced no interference provided that the sample was heated for no less than 2 minutes in the water bath. The presence of more than 2 ml of the concentrated acid produced a decrease in the optical density of the solution.

Solutions that had been fumed with sulphuric acid and treated by the above procedure gave very low and erratic results unless they had been boiled vigorously with hydrobromic acid before adding the reagent. The following procedure was found satisfactory for samples containing not more than 2 ml of concentrated sulphuric acid.

Cool the iridium solution after fuming and add 5 ml of concentrated hydrobromic acid. Boil this solution vigorously in a small beaker covered with a watch-glass until all but 1 or 2 ml of the hydrobromic acid has been expelled. Cool this solution and transfer it to a 20-ml test-tube, rinsing with 5 ml of concentrated hydrobromic acid. Continue with the recommended procedure described above.

The mean optical density of six replicate fumed samples, each containing 56·7 μg of iridium in a final volume of 25 ml, was 0·598. The mean deviation of these results was 0·003 or 0·5 per cent.

EFFECT OF DIVERSE IONS—

Platinum, rhodium and palladium chlorides, treated by this procedure, gave absorption spectra as shown in Fig. 2. The rhodium colour is not stable, and attempts to develop a precise determination for rhodium with the possibility of a simultaneous determination of rhodium and iridium in solution were unsuccessful.

The platinum spectrum shows a quite reproducible and stable peak at 455mμ. Four replicate samples each containing 8·0 μg of platinum per ml showed optical densities of 0·390, 0·390, 0·389 and 0·380. This represents an increase in sensitivity over the corresponding procedure with stannous chloride in hydrochloric acid,[10] so that further investigations of this reaction may prove worth while. The absorption minimum in the neighbourhood of 400 mμ did not prove to be reproducible, and attempts to produce a simultaneous determination for iridium and platinum in solution were abandoned after a few trials.

Palladium produces a light brown colour with an absorption peak at 385 mμ. This colour is neither reproducible nor stable.

All substances that oxidise hydrobromic acid interfere in this procedure unless the precaution is taken to expel the bromine thus produced. This can be easily accomplished by boiling the solution with 5 ml of hydrobromic acid in a procedure similar to that described above for sulphuric acid solutions. In this manner several samples of iridium in solutions containing nitric acid were successfully analysed.

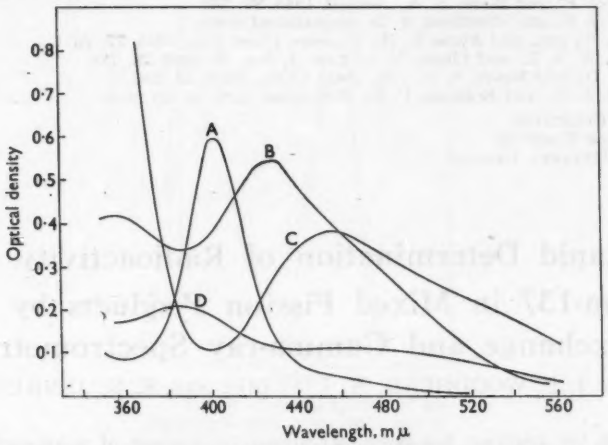

Fig. 2. Spectral curves for iridium, rhodium, platinum and palladium: curve A, 2·26 p.p.m. of iridium; curve B, 2·03 p.p.m. of rhodium; curve C, 8·06 p.p.m. of platinum; curve D, 2·02 p.p.m. of palladium

Various ions that may be associated with iridium solutions were deliberately added to test their effect on the procedure. The concentrations of these ions necessary to produce a 1 per cent. deviation in optical density are as follows—

Ion	Cu^{++}	Ni^{++}	Co^{++}	Fe^{++}	Sb^{+++}	Cr^{+++}	Ti^{++++}	Au^{+++}
Tolerance for a 1 per cent. deviation in optical density, p.p.m.	40	20	10	16	80	13	6	precipitate

It should be noted that all interfering substances tested, with the exception of titanium, produced a decrease in the optical density of the solution.

The iridium solution to be tested must initially be no more than 0·5 *M* in hydrochloric acid.

DETERMINATION OF IRIDIUM AFTER REMOVAL OF OTHER PLATINUM METALS—

Westland and Beamish have recently suggested a separation of very small quantities of rhodium and iridium by precipitation of the former from sulphuric acid solution by means of antimony.[11] The determination of the iridium in the residual solution as described by these authors is tedious. We have found that the procedure described in this paper may be successfully applied to portions of the iridium solution. The relatively large amount of antimony present in solution produced an appreciable light absorption under the conditions of the iridium determination, but this diminished to a reasonably small value when half the samples were taken for analysis. Evidently the optical density due to antimony in hydrobromic acid solution is not directly proportional to the antimony concentration. For all determinations of iridium under these conditions a blank was prepared by putting a sample containing no iridium through the entire procedure. In our work this blank amounted to the equivalent of about 2·6 μg of iridium.

Determinations of iridium in artificial samples from which the five other platinum metals had been removed, the immediately previous stage in the separation being the precipitation of rhodium with antimony, gave the following results—

Iridium taken, μg	7·8	716	1·95	1·95
Iridium found, μg	7·4	720	1·90	1·90

We are grateful for assistance in the form of a grant from the Ontario Research Foundation.

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March 28th, 1956

The Rapid Determination of Radioactivity due to Caesium-137 in Mixed Fission Products by Anion Exchange and Gamma-ray Spectrometry

BY J. L. WOODHEAD, A. J. FUDGE AND E. N. JENKINS

An investigation has been carried out on the use of anion-exchange resins in the hydroxide and carbonate forms as solid precipitants in the analysis of mixed fission-product solutions for caesium-137. The method described involves the isolation of caesium-137, a De-Acidite FF anion-exchange resin column in the carbonate form being used as the solid precipitant for the removal of other fission-product elements. Caesium-137 is determined by measurement of its barium-137 daughter on a gamma scintillation spectrometer.

The method compares favourably with a recent procedure for the determination of caesium by gamma spectrometry and with a standard complete radiochemical separation procedure.

THE current methods for the analytical separation of individual fission-product elements rely on the repetition of a number of chemical steps, such as precipitation, solvent extraction or distillation, to give the required high degree of chemical purity.

Gamma spectrometry makes it possible to distinguish by means of their energies the various components of a mixture of gamma emitters.[1,2] Individual components of a simple mixture of gamma emitters can often be determined quantitatively by means of gamma spectrometry without prior chemical separation.

The measurement of caesium-137 in fission-product mixtures is frequently required, *e.g.*, as a monitor for the determination of the consumption of nuclear fuel. The caesium-137 is in equilibrium with a short-lived daughter, barium-137*m*, which emits a 0·66-MeV gamma ray. In principle the caesium could be determined very rapidly by measuring the height of the photopeak response at 0·66 MeV, with a sodium iodide (thallium activated) scintillation spectrometer. Unfortunately the caesium-137 is often accompanied by products emitting gamma rays of similar or of higher energy—notably niobium-95 (0·74 MeV), zirconium-95 (0·72 MeV), ruthenium-106 (0·62 MeV and 0·51 MeV) and lanthanum-140 (1·60 MeV and 0·82 MeV). A typical example of interference by zirconium-95 - niobium-95 is shown in Fig. 1.

The general advantages of a gamma-spectrometric method would largely be preserved if the caesium could rapidly and simply be removed from most of the interfering elements.

Kittle[3] separated interfering elements by two ferric hydroxide scavenges, finally precipitating the caesium as chloroplatinate. This method of removal of the bulk of the impurity by scavenging followed by a moderately specific precipitation of the element to be

determined necessitates the addition of a carrier initially and the determination of the chemical yield of the final precipitate. The alternative purification described in this paper is quantitative and demands the minimum of manipulation.

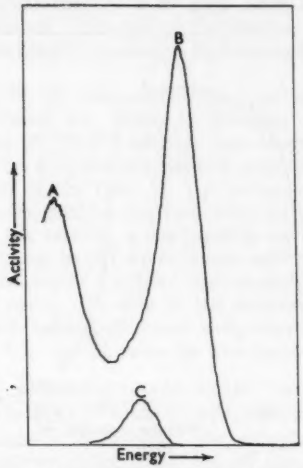

Fig. 1. Gamma spectra of unseparated and separated fission-product solutions: A, ruthenium-106 photopeak, 0·51 MeV; B, zirconium-95 - niobium-95 photopeak, 0·73 MeV; C, caesium-137 photopeak, 0·66 MeV, after separation from mixed fission products

The use of an anion-exchange resin as a solid precipitant and filter was first suggested by Glueckauf and Roberts[4] and further reported by Samos and Finston[5] and also by Welch[6,7]. Glueckauf and Roberts carried out qualitative tests on the precipitation of tracer and milligram amounts of various elements on De-Acidite FF anion-exchange resin in the hydroxide, carbonate, sulphide and chromate forms. Samos and Finston used Dowex 1 in the chloride form for the separation of milligram amounts of bismuth and thorium, the bismuth being precipitated on the column as oxychloride and the thorium eluted. They also separated strontium and yttrium by precipitation of the latter on a hydroxide column. The columns were shown to retain in both cases at least 99 per cent. of the precipitated element. Welch has used an anion-exchange resin in the hydroxide form to separate strontium and caesium from fission-product solutions, and has done qualitative work on trace amounts of materials on columns in other forms.

The method now described involves the isolation of caesium-137, an anion-exchange resin column in the carbonate form being used as a solid precipitant for the removal of other fission-product elements in the solution. The gamma spectrometer is used to measure the caesium-137 activity. The method is compared with the method of Kittle and the relative merits of each technique for various fission-product solutions are assessed.

Experimental

Behaviour of caesium on an anion-exchange resin in the hydroxide form—

By using an anion-exchange resin in the hydroxide form, it should be possible to remove by precipitation the fission product with relatively insoluble hydroxides, and any anionic species present. These in fission-product solutions that had stood for more than 30 days after irradiation would be zirconium, niobium, cerium, lanthanum and the rare-earth activities as insoluble hydroxides, and possibly ruthenium as an anionic complex. This would leave caesium, strontium and barium in solution.

A column of anion-exchange resin, De-Acidite FF (60 to 100 mesh size; 0·25 g) in the hydroxide form was made up in demineralised water. A carrier-free solution of caesium-137 at pH 6 was applied to the column, which was washed with demineralised water at a flow rate of 0·1 ml per minute. Fig. 2 is an elution graph showing the recovery of the caesium.

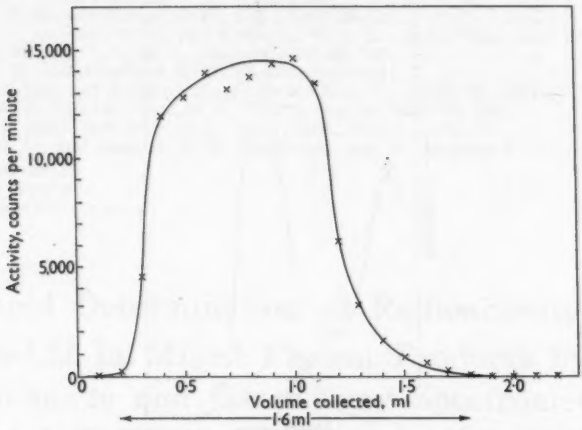

Fig. 2. Elution of caesium-137 from a column of anion-exchange resin in the hydroxide form; 0·25 g of 60 to 100-mesh De-Acidite FF in a 2-cm long column; rate of flow through column, 0·1 ml per minute

BEHAVIOUR OF MIXED FISSION PRODUCTS ON AN ANION-EXCHANGE RESIN IN THE HYDROXIDE FORM—

A carrier-free solution of uranium-235 fission products of several months irradiation, that had been standing for about 5 months, was put on a column of De-Acidite FF (60 to 100 mesh; 0·25 g) in the hydroxide form. The acidity of the solution had been reduced to 0·01 *M* and the uranium removed by solvent extraction. A gamma spectrum on the first 2 ml of effluent showed a peak at 0·66 MeV due to caesium-137, and at 0·14 MeV due to cerium-144. The cerium break-through was not expected and its behaviour was investigated further.

BEHAVIOUR OF CERIUM ON AN ANION-EXCHANGE RESIN COLUMN—

A solution of carrier-free cerium-144 - praseodymium-144 as supplied by the Radiochemical Centre, Amersham, was put through a further purification procedure.[8] A portion of the purified solution was added to a carrier solution of cerium, and exchange was ensured by a series of oxidation and reduction cycles.

Initial experiments with carrier-free cerium^{III} on a hydroxide column showed that very little activity was retained on the resin, and that the amount being retained increased with the age of the resin, which had been dried and stored in open vessels. The following table shows the percentage of cerium retained by the resin during a period of a week, after preparation in the hydroxide form—

Time, days	2	4	6	8
Cerium retained, % ..	10	40	70	90

At the end of the period of storage, it was noted that carbon dioxide was evolved from the resin on treatment with dilute mineral acid. Hence the resin was acting as a solid absorbent for carbon dioxide from the air and it appeared that the retention of cerium^{III} was directly related to the carbon dioxide content of the resin.

A sample of resin in the hydroxide form was then prepared and care was taken to prevent the resin from coming into contact with carbon dioxide. Storage was under freshly boiled water in an atmosphere free from carbon dioxide. The retention of cerium^{III} on this hydroxide-form resin was tested with a solution containing carrier and tracer cerium. No cerium was found to be retained on the column. The solution was, however, low in total

anion concentration, and the pH value of the effluent containing the cerium was found to be about 10. Potentiometric titration of the untreated carrier solution showed that precipitation of cerous hydroxide occurred at pH 7·7.

Increasing the anion content of the cerium solution to at least molar in chloride ion, which should give molar concentration of hydroxide after exchange, caused precipitation of the cerium to occur on the column. The pH of the effluent was greater than 11, indicating that a large excess of anions is necessary to ensure a high enough concentration of free hydroxyl ions to precipitate the cerium.

The behaviour of cerium on an anion-exchange resin column in the carbonate form was tested under similar conditions, *i.e.*, molar in chloride. Complete precipitation of the cerium^{III} occurred, although the pH of the effluent was never more than 8.

The behaviour of cerium on a hydroxide resin is anomalous and an explanation is not easily seen. The decomposition of the resin by the action of alkali to give an alkylamine (one of the condensation products used in the formation of the resin), which is then acting as a weak complexing agent on the cerium, is one possible explanation. The complex would, if formed, be weak, since an increase in pH would cause hydrolysis to take place. One other possible explanation is the formation of a colloid that is stable in near neutral solutions but that coagulates at a higher pH value. In view of the anomalous behaviour of cerium on the hydroxide column and the predictable behaviour with resin in the carbonate form, it was decided to investigate further the use of resin in the carbonate form.

BEHAVIOUR OF BARIUM ON AN ANION-EXCHANGE RESIN COLUMN—

As is to be expected, the solubility of barium hydroxide is too high to cause precipitation on a resin column in the hydroxide form under the conditions employed. The solubility of barium carbonate is considerably lower than that of the hydroxide so that retention of the barium would be expected on an anion-exchange resin column in the carbonate form. Barium showed one anomalous characteristic on such a column. When a solution containing barium carrier and barium-140 - lanthanum-140 tracer was applied to a 2-g column of De-Acidite FF (60 to 100 mesh size) in the carbonate form, precipitation occurred, as shown by a white band on the column. On washing with demineralised water, activity was found in the effluent, as shown in Fig. 3. Increasing the amount of carrier barium to twice its

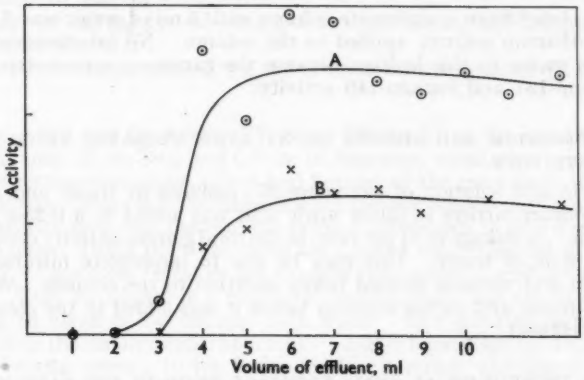

Fig. 3. Elution of barium from a carbonate column; 1-ml fraction; demineralised water as eluting agent: curve A, 10 mg of barium on column; curve B, 20 mg of barium on column

original amount (20 mg) and using the same activity of tracer showed that the elution of barium had been cut to one-half of its original rate. This indicated some form of solution of the barium. It is unlikely that this is simple solution of barium carbonate, since the solubility of barium carbonate has been shown by Teicher[9] to be 0·5 mg per litre at a pH of about 9·5. Teicher also observed that as the pH decreased the solubility of barium carbonate increased to at least ten times this latter value at pH 7. This was possibly due to the reaction—

$$BaCO_3 + CO_2 + H_2O \rightarrow Ba(HCO_3)_2$$

Under the conditions of this experiment a drop in pH from 10 to 7 would occur as the column is washed with water. This drop was observed by measuring the pH of the effluent and also the activity corresponding to this change. The activity removed from the column by washing with water was shown to be pure barium-140 by a determination of the energy of the gamma rays (see Fig. 4). No energy greater than 0·54 MeV was found, showing that lanthanum-140 was retained completely on the column.

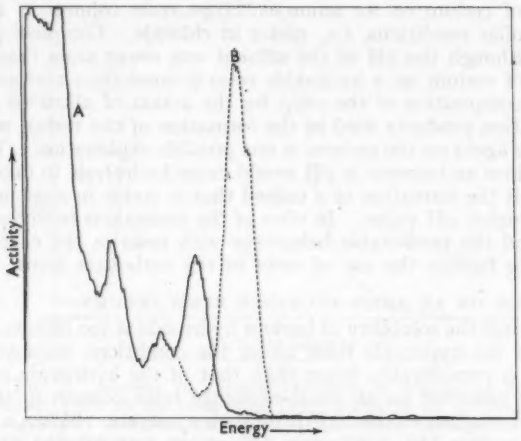

Fig. 4. Gamma spectrum of barium-140 from carbonate column: curve A, separated barium spectrum; curve B, standard caesium-137 photopeak, 0·66 MeV

The activity eluted from a carbonate column with 5 ml of water was 5 per cent. of the total barium - lanthanum activity applied to the column. No interference would result in the determination owing to this leakage because the gamma spectrometer can successfully resolve the caesium-137 and barium-140 activity.

BEHAVIOUR OF ZIRCONIUM AND NIOBIUM ON AN ANION-EXCHANGE RESIN COLUMN IN THE CARBONATE FORM—

A 0·5 *M* nitric acid solution of zirconium-95 - niobium-95 tracer and about 0·1 mg of zirconium and niobium carriers in dilute nitric acid was added to a 0·25-g resin column in the carbonate form. A leakage of 2½ per cent. of the total gamma activity occurred on washing the column with 5 ml of water. This may be due to incomplete filtration of the radio-colloidal zirconium and niobium formed before addition to the column. When oxalic acid was added to the tracer and carrier solution before it was added to the column, no activity was found in the effluent.

BEHAVIOUR OF RUTHENIUM ON AN ANION-EXCHANGE RESIN IN THE CARBONATE FORM—

Ruthenium is not precipitated when a solution of the nitrosotrinitrate is made alkaline with sodium hydroxide or sodium carbonate. It is known to exist in nitric acid solution as anionic species and would thus be retained on an anion-exchange resin column provided the concentration of other anions is not too great. This predicted behaviour has been established in practice with a resin column in the carbonate form.

A solution containing tracer and carrier ruthenium nitrosotrinitrate in 6·5 *M* nitric acid was diluted to reduce the acidity to 0·5 *M*. Less than 0·2 per cent. of the activity appeared in the first 5 ml of eluant when applied to a resin column in the carbonate form and washed with water. When this solution was made 6 *M* in sodium nitrate and applied to a resin column in the carbonate form, no activity was retained by the column on washing with 5 ml of water.

BEHAVIOUR OF CAESIUM ON AN ANION-EXCHANGE RESIN COLUMN IN THE CARBONATE FORM—

When a solution containing caesium tracer and carrier was applied to an anion-exchange resin column in the carbonate form, complete elution occurred in the first 2 ml of effluent. A solution of fission products was applied to a resin column in the carbonate form and the column was washed with 5 ml of water. The resin was subjected to wet oxidation with nitric and perchloric acids, followed by a radiochemical separation after the addition of carrier. Only 0·5 per cent. of the original caesium activity was found to have remained on the resin.

METHOD

PREPARATION OF RESIN—

Normal grade De-Acidite FF was dried for 16 hours at 100° C. The resin was then ground in a coffee-mill and graded into − 80 and + 100 B.S. sieve size. This fraction was then made into a column, 12 inches × 1 inch, by pouring into water slowly and allowing to settle out. A 3 *M* solution of sodium carbonate was then allowed to flow through the resin at a rate of about 10 ml per minute until ten column volumes (approximately 250 ml) had passed through. The column was then washed with twenty column volumes (approximately 500 ml) of demineralised water, and the resin was poured into a stoppered bottle and stored under demineralised water.

PREPARATION OF CARRIERS—

Caesium carrier (50 *mg per ml*)—7·33 g of pure caesium nitrate were dissolved in 100 ml of demineralised water.

Ruthenium carrier—2·04 g of Specpure ruthenium trichloride were dissolved in an alkaline solution of potassium periodate. The solution was stirred with an equal volume of carbon tetrachloride and during the stirring was made acid with concentrated sulphuric acid. The carbon tetrachloride phase was separated and washed once with water, and it was then treated with 30 ml of 8 *M* nitric acid. The nitrous fumes produced by the action of 8 *M* nitric acid on copper turnings were passed into the mixture until no more ruthenium was extracted. The nitric acid solution was then boiled to remove excess of nitrous fumes, cooled and diluted to 100 ml with water.

Zirconium carrier (10 *mg per ml*)—3·0 g of zirconyl nitrate and 1·0 g of oxalic acid were dissolved in 100 ml of water.

Barium carrier (10 *mg per ml*)—1·9 g of barium nitrate was dissolved in 100 ml of water.

PROCEDURE—

Make a slurry of 1 g of De-Acidite FF (80 to 100 mesh), in the carbonate form, and put it into a glass column, 25 cm long and 0·7 cm in diameter, fitted with a well ground tap and a fine tip. Use cotton-wool plugs at top and bottom of the resin bed. Initially 1 g of dry resin was made into a column and the dimensions of the wet column were measured. Sufficient resin in the carbonate form was put into the column afterwards to give a similar size column, *i.e.*, about 7 cm long. Drain the water from the column until the level is just above the top cotton-wool pad. Care must be taken not to allow the column to run dry at any time during the procedure. Take an aliquot of a fission-product solution, and adjust its acidity to pH 1 by the addition of alkali or by dilution with water. The salt concentration must not be greater than 3 *M* in the finally prepared sample. Add 0·1-ml aliquots of caesium, ruthenium, zirconium and barium carriers to the fission-product solution, and add 1 ml of the mixture to the resin column. Allow the fission product solution to run into the resin and collect the effluent at a rate of not more than 6 drops per minute (0·3 ml per minute) in a small polythene cup. The dimensions of the cups used are: internal diameter, 2·5 cm; external diameter, 3·5 cm; height, 2·0 cm; base thickness, 0·1 cm. These were turned from polythene rod.

Stir the solution with a thin polythene rod. Stand the cup on a 250 mg per sq. cm aluminium absorber on the aluminium housing of a sodium iodide (thallium activated) crystal of a gamma scintillation spectrometer (*e.g.*, A.E.R.E. type 1387A). Determine the peak height of the photopeak at 0·66 MeV due to caesium-137. Determine the peak height of 5 ml of a 4-π counted caesium-137 solution in a similar cup under identical geometry.

The disintegration rate of the caesium-137 in the fission-product mixture is then calculated from the peak heights.

Results

In order to test the method for the analysis of caesium, a series of solutions containing fission products were obtained or synthesised from separated individual isotopes. These solutions, six in all, were than analysed by the use of an anion-exchange resin column in the carbonate form, and also by the method of Kittle, in which scavenges with ferric hydroxide and final precipitation as chloroplatinate are used. In several cases also the solutions were analysed for radiochemical caesium by a standard radiochemical purification procedure and determined as solid sources on a gamma spectrometer.

A purified solution of caesium-137 (carrier free) was always analysed together with the sample, and the results are expressed as a ratio of sample to standard. A check was also made against an untreated aliquot of this solution, which was diluted to 5 ml and measured under similar geometry. The results in Table I are the mean of several determinations in each case.

Table I

Analysis of fission-product solutions for caesium-137

Fission product solution No.	Acidity	Uranium concentration	Activity				Ratio of sample to standard solution by—		
			^{95}Zr + ^{95}Nb*	^{140}Ba + ^{140}La*	^{106}Ru*	^{144}Ce*	Radiochemical purification method	Kittle method	Resin method
1	3 *M* HNO_3	0·1 mg per ml	1	–	1	2	0·96	0·99	0·93
2	4 *M* HNO_3	0·1 mg per ml	not known	not known	not known	not known	2·86	2·86	2·89
3	6 *M* HNO_3	25 mg per ml	0·5	–	0·7	1	1·52	1·54	1·46
4	0·01 *M* HNO_3	10 μg per ml	5	1	3	3	not measured	3·96	3·93
5	0·1 *M* HCl	not known	0·3	0·5	0·1	1	not measured	2·49	2·52
6	9 *M* H_2SO_4	15 mg per ml + 10 mg of Zr per ml	not known	not known	not known	not known	0·67	0·68	0·69

* These activities are expressed as an approximate ratio to the caesium-137 activity of the solution as measured on a scintillation counter.

The decontamination factors for zirconium plus niobium and ruthenium were calculated from spectra obtained before and after column treatment (see Fig. 1). The decontamination factor for zirconium-95 plus niobium-95 was at least 200 and for ruthenium-106 about 100. The contribution of these activities to the caesium-137 - barium-137 photopeak is, therefore, very small.

For the analysis of solutions 3 and 6 the method of Kittle had to be modified because of the interference of the sodium, added as sodium hydroxide to neutralise excess of acid and to precipitate the ferric hydroxide, on the chemical yield of the caesium as chloroplatinate. After the ferric hydroxide step the caesium was precipitated by means of sodium cobaltinitrite reagent. This reduces the concentration of sodium cation considerably. The caesium cobaltinitrite was then dissolved in a few drops of concentrated nitric acid, and the caesium was precipitated as the chloroplatinate.

Conclusions

The use of an anion-exchange resin as a solid precipitant to separate interfering elements in the determination of caesium by using a gamma spectrometer has much to recommend it. An examination of the results shows that they are as accurate as those obtained by a classical complete radiochemical separation. The positive advantages of using a resin column for precipitation before the gamma-spectrometric analysis of fission-product solutions are—

(*a*) The transference of solutions is greatly reduced, which minimises losses of active material. By this means losses of caesium have been eliminated so that the determination of chemical yield becomes unnecessary. This leads to a saving in handling time.

(*b*) Provided adequate precautions are taken for the storage of the resin after preparation, it is a stable reagent. The activity can be removed by acid washing, and the resin can be regenerated with carbonate.

(*c*) The amount of apparatus involved is very small and it can easily be decontaminated.

The use of an ion-exchange resin as a solid precipitant in other chemical forms can be extended to include not only its use as a means of removing interfering activities, but also to retain the element to be analysed, the interfering elements being washed out of the column. One difficulty of this type of system is the contamination of the precipitate by anionic species in the solution, *e.g.*, ruthenium. This could be overcome by increasing the anion content of the solution added to the resin.

This type of column could also be applied to rapid group separations such as chlorides, sulphides, hydroxides, oxalates, sulphates and so on, not only removing the unwanted elements but also to isolate groupwise the elements before direct gamma-ray assay of the resin phase.

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May 9th, 1956

Quantitative Inorganic Chromatography

Part II.* The Determination of Vanadium in the Presence of Excess of Dichromate

By F. H. POLLARD, G. NICKLESS and A. J. BANISTER

The difficulty of determining vanadium in the presence of excess of dichromate is overcome by separating the vanadium from dichromate by paper chromatography. After separation, the vanadium is extracted from a section of the chromatogram and determined by colorimetric methods. The dichromate is partly reduced to chromic salt by the paper during the elution and moves with the vanadium, but the amount of chromium then associated with the vanadium is insufficient to interfere with the colorimetric analytical procedures used.

A new chromatographic spray reagent (sodium 2-nitroso-1-naphthol-4-sulphonate) is also described for the detection of Cu^{II}, Au^{III}, U^{IV}, Fe^{III}, Fe^{II}, Co^{II}, Ni^{II}, Pd^{II} and Pt^{IV}.

The determination of vanadium in the presence of chromium or its salts is not easily accomplished by existing chemical methods when the ratio of vanadium to chromium is of the order of 1 to 100. Interference in the volumetric or colorimetric methods occurs because of the intense colour of chromium salts, while gravimetric methods have not been found applicable. The basis of the method described in this paper is the chromatographic separation of the vanadium from the dichromate, but, owing to the reduction of dichromate by the filter-paper to the chromic ion, some chromium still remains associated with the vanadium

* For particulars of Part I of this series, see reference list, p. 581.

after the chromatographic elution. By these means the vanadium to chromium ratio can be increased to approximately 1 to 15 or greater, which is more favourable for the already established colorimetric methods for vanadium. The procedure can therefore be regarded as a chromatographic enrichment technique carried out before the determination.

Sandell[1] used a modification of the method due to Montequi and Gallego[2] for the determination of vanadium in silicate rocks. Vanadium was extracted as the 8-hydroxyquinolate with chloroform from a weakly acid aqueous solution (pH 4) of vanadate and dichromate. Direct colorimetric analysis of the chloroform extract gave irreproducible results, although Talvitie[3] found that Beer's law was obeyed if the chloroform was free from alcohol. Since iron is present in appreciable quantities in Whatman 3MM paper, used for the chromatographic procedure, Talvitie's method was not considered suitable in our work, since the iron 8-hydroxyquinolate formed with the paper extract would have strongly interfered with the vanadium determination. Instead of the direct colorimetric determination, Sandell found it better to remove the chloroform by evaporating and to fuse the residue with sodium carbonate. The resulting sodium vanadate was then determined as the yellow complex acid formed with sodium tungstate in phosphoric acid solution.[4] A more sensitive method has recently been suggested by Tanaka,[5] in which vanadate is reduced to vanadyl and then determined as the yellow complex with formaldoxime. In the work described in this paper, both Sandell's and Tanaka's methods have been used for the final determination of vanadium.

EXPERIMENTAL

Chromatographic separation of vanadium, as vanadate, from chromium as dichromate

An all-glass apparatus for downward chromatography consisted of two troughs (24 cm long and 2·5 cm in diameter) supported by a glass stand contained in a tank (23 cm × 28 cm and 52 cm deep) covered with a ground-glass plate drilled with two holes located one over each of the two troughs. The purpose of the holes was to permit solvent to be admitted into the trough without removing the glass cover-plate and so disturbing the equilibration of the tank.

The solvent mixture used for both equilibrating the tank and for eluting the chromatogram consisted of 15 ml of water, 4 ml of AnalaR concentrated hydrochloric acid, 50 ml of diethyl ether and 30 ml of methanol.[6] Some solvent mixture was put in the bottom of the tank (about 150 ml) and also in the first trough and left for 24 hours to equilibrate the tank.

(The Whatman 3MM paper to be used for the chromatographic separation was purified by acid washing for 6 days and then washed free from chloride.[6]) A sheet of filter-paper bearing the wet band of the mixture to be separated was placed in position in the second trough and then left for a further 4 hours to re-equilibrate. At the end of this time, a quantity of the solvent mixture was admitted to this second trough, which up to now had been empty. The chromatogram was run for approximately 3 hours at room temperature (17° ± 2° C), and the solvent front travelled a distance of 30 cm from the starting line.

After removal of the chromatogram from the tank and drying, it was sprayed with the reagents described below. The vanadium was detected at R_F 0·5, dichromate at R_F 0·8 and the Cr^{III} from the reduction of dichromate by the paper at an R_F of 0·5, *i.e.*, in the same position as the vanadium.

Spray reagents used—

Tannic acid solution—0·5 g of tannic acid and 1 g of sodium acetate in 10 ml of warm 60 per cent. industrial methylated spirit. With this reagent vanadium gave a dark blue colour.[7]

Sodium 2-nitroso-1-naphthol-4-sulphonate solution—0·05 g of reagent in 100 ml of a water - ethanol mixture (1 + 1 v/v). This reagent gave a deep pink brown colour with dichromate after exposure to ammonia vapour.

Note—The use of this reagent has been described previously by Sarver[8] for the detection of copper, cobalt and ferrous ions. Wise and Brandt[9] have also used it for the spectrophotometric determination of cobalt. Since the use of the latter reagent has not been previously described for the chromatographic purposes, it should be noted that it serves as a sensitive spray reagent for the chlorides of the following: Cu^{II} (orange-pink), Au^{III} (orange-brown), U^{IV} (violet), Fe^{II} and Fe^{III} (if in low concentration, green;

high concentration, brown), Co^{II} (rose), Ni^{II} (yellow), Pd^{II} (red-violet), Pt^{IV} (brown), and Cr^{III} and Cr^{VI} (pink-brown; intensity increasing on keeping for a few days).

Determination of vanadium

Preparation of chromatogram—

Two types of quantitative chromatogram were investigated—

(*i*) *The wide paper strip*—A sheet of paper 20 cm × 45 cm was used for the chromatogram,[10] with the starting line marked 10 cm from the upper narrow edge. The solution to be analysed was applied as a band 12 cm × about 1·5 cm extending about 0·75 cm above and below the starting line and to within 4 cm of each edge. Spots of the solution (approximately 0·02 ml, giving spots of diameter about 1·5 cm) were placed 3 cm from the ends of the band. After elution, strips 3 cm wide were cut from the sides of the chromatogram and sprayed for vanadium with tannic acid. With these pilot strips as a guide, the vanadium-bearing section of the paper was cut from the main chromatogram and used for the determination.

(*ii*) *The twin paper strips*—The starting line was marked 8 cm from and parallel to one of the short edges of a sheet of paper 35 cm × 20·5 cm. Starting from the opposite edge, a slot 29 cm long and 0·5 cm wide was cut from the centre of the paper. This divided the chromatogram into twin strips 10 cm wide and 29 cm long, which were joined for 6 cm above the starting line. Identical volumes of the solution to be analysed were applied to each strip. One strip was sprayed to locate the vanadium and the other was used for the determination.[11]

After the initial trial experiments, the second type of chromatogram was chosen for the subsequent quantitative work, for the following reasons—

(*i*) Since the twin paper strips were eluted under almost identical conditions, the position of the vanadium on the pilot strip gave a more reliable indication of the position of the vanadium on the strip used for the analysis.

(*ii*) With the wide paper chromatograms and low vanadium to chromium ratios, the vanadium on the pilot strips was insufficient for detection. To overcome this difficulty, it was necessary to use a solution with a higher ratio of vanadium to chromium for the pilot strips. This gave a less reliable indication of the position of the vanadium in the centre of the chromatogram. Further details on the variation of R_F with concentrations of ions, etc., will be given in a later paper on the quantitative determination of ferrous and ferric iron.

For each vanadium to chromium ratio, chromatograms were run containing 50 μg of vanadium. The solution was applied to the starting line from an Agla micrometer syringe, and to preserve a band about 1·5 cm wide two or more applications were necessary when the vanadium concentration was low; the paper was allowed to dry (without warming) before the next application.

Various standard solutions were prepared, from AnalaR ammonium vanadate and AnalaR potassium dichromate, containing the following ratios of vanadium to chromium: 1 to 1, 1 to 10, 1 to 100 and 1 to 200. After elution and drying, the vanadium was located upon the pilot strip with tannic acid, and the vanadium-bearing section on the unsprayed strip was cut out.

This section was cut up, ignited in a platinum crucible, the residue was fused with excess of sodium carbonate, and the melt was extracted with water. The aqueous extract (volume 4 to 5 ml) was neutralised by dropwise addition of *M* sulphuric acid from a capillary pipette, until the solution gave the intermediate (orange) colour with methyl orange indicator. The solution was buffered to pH 4 with 5 ml of the standard buffer of sodium acetate and acetic acid (0·036 *M* sodium acetate in 0·164 *M* aqueous acetic acid). The solution was put into a 30-ml stoppered test-tube (ground-glass stopper). To this was added 0·5 ml of 2 per cent. 8-hydroxyquinoline in 10 per cent. v/v aqueous acetic acid and 3 ml of chloroform. The mixture was shaken for 1 minute, and the layers were allowed to separate, the vanadium being extracted into the lower layer as the chloroform-soluble red-brown complex. The lower layer was separated off by means of a capillary pipette (fitted with a rubber teat as is commonly employed for transferring small volumes of liquid) and the extraction procedure was repeated once more. The extracts and pipette washings were placed in the platinum

crucible; after evaporation of the chloroform, 0·05 g of anhydrous AnalaR sodium carbonate was added, and the mixture was fused, cooled and extracted with water. This extract was then analysed by one of the methods given below.

COLORIMETRIC DETERMINATION BY SODIUM TUNGSTATE METHOD—

Reagents—

Sodium tungstate, 0·05 M—16·5 g of AnalaR sodium tungstate dissolved in 100 ml of water.

Nitric acid, 0·5 M.

Phosphoric acid, diluted—One volume of orthophosphoric acid, sp. gr. 1·75, mixed with two volumes of water.

Standard vanadium solution (0·100 *mg of vanadium per ml*)—0·2295 g of AnalaR ammonium vanadate dissolved in a mixture of 100 ml of water plus 7·5 ml of concentrated nitric acid, sp.gr. 1·42, and the solution diluted to 1 litre.

Calibration of spectrophotometer for vanadium—A series of standard colorimetric solutions containing 0 to 10 μg of vanadium per ml was prepared as follows. An appropriate volume of standard vanadium solution was put in a 10-ml calibrated flask, and to this were added 2·0 ml of 0·5 *M* nitric acid and 2·0 ml of diluted phosphoric acid and then 0·5 ml of 0·05 *M* sodium tungstate; the solution was diluted to the mark with distilled water.

The optical densities of these standard solutions were measured at 410 mμ with a Unicam spectrophotometer, 10-mm glass cells fitted with lids being used. Beer's law was obeyed over the range 0 to 10 μg of vanadium per ml, and the slope of the absorbing curve was 0·047 optical-density units per μg of vanadium per ml.

Quantitative determination of extracts from chromatograms—By using the calibration curve and the procedure described above, the vanadium contents were determined after chromatographic separation of the mixtures containing various proportions of vanadium to chromium.

TABLE I

DETERMINATION OF VANADIUM BY SODIUM TUNGSTATE METHOD

Ratio of vanadium to chromium	Vanadium present, μg	Vanadium found, μg
1 to 1	50·0	50·5, 50·2
1 to 10	50·0	50·5, 50·4, 50·5
1 to 100	50·0	50·4, 50·5, 50·0
1 to 200	50·0	50·4, 50·0, 50·9

Results—The results were all expected to be on the high side, since distilled water and not a paper blank was used, thus taking no account of the impurities left in the paper after acid washing. Nevertheless, the results in Table I show that 50 μg of vanadium as vanadate in the presence of up to 200-fold excess of chromium as dichromate can be determined to within 2 per cent. in a single determination. However, in order to place the full quantity of vanadium - chromium mixture on the starting line as a thin band (about 1·5 cm), three or four applications from the syringe were necessary. Between each application the band was allowed to dry, and during this time reduction of dichromate to chromic salt by the cellulose took place. Since chromium and vanadium have close R_F values and chromium interferes in the final vanadium determination, a more sensitive method of determination was investigated. This was the reaction between the vanadyl ion and formaldoxime in ammoniacal solution to give a yellow colour. According to Tanaka, Beer's law is obeyed over the range 0 to 10 μg of vanadium per ml. In the results for the following section, various known quantities of vanadium were used and these are listed.

COLORIMETRIC DETERMINATION BY FORMALDOXIME METHOD—

Reagents—

Hydrochloric acid, 0·2 M—Prepared from AnalaR hydrochloric acid.

Formaldoxime hydrochloride solution—2·5 g of formaldoxime hydrochloride in 100 ml of water.

Ammonia solution, sp.gr. 0·880—AnalaR.

Standard vanadium solution—As above.

Sodium metabisulphite.

Calibration of spectrophotometer for vanadium—A series of standard colorimetric solutions containing from 0 to 5 μg of vanadium per ml was prepared as follows. An appropriate volume of standard vanadium solution was placed in a 25-ml conical flask, and 5·0 ml of 0·2 *M* hydrochloric acid together with 0·05 g of sodium metabisulphite were added. The solution was then evaporated to a few drops to reduce the vanadate to vanadyl and to remove the excess of sulphur dioxide. The solution was cooled and transferred to a 10-ml calibrated flask; the conical flask was rinsed with water, and the washings were added to the calibrated flask. To this solution were added 2 ml of formaldoxime hydrochloride solution and 1 ml of ammonia solution, and the mixture was diluted to the mark with water. The optical density of the yellow colour so produced was measured, after 30 minutes at 410 mμ with the Unicam spectrophotometer, 10-mm glass cells being used. The slope of the absorbing curve was found to be 0·136 optical-density units per μg of vanadium per ml, and Beer's law was obeyed over the range 0 to 5 μg of vanadium per ml, which confirms Tanaka's results. This method for the final analysis made it possible to use one-fifth of the quantity previously used for chromatographic separation.

Quantitative determination of extracts from chromatograms—The procedure was similar to that adopted in the sodium tungstate method as far as the final fusion with sodium carbonate. The residue was then dissolved in water, transferred to 25-ml conical flask, 0·05 g of ammonium persulphate was added, and the solution was evaporated to dryness. To destroy the excess of persulphate, the residue was evaporated to dryness with 5 ml of AnalaR concentrated hydrochloric acid. This residue was then treated as for the preparation of the calibration graph described above, and the vanadium content was determined from the calibration graph.

Results—Results are shown in Table II.

TABLE II

DETERMINATION OF VANADIUM BY FORMALDOXIME METHOD

Ratio of vanadium to chromium	Vanadium present, μg	Vanadium found, μg
1 to 1	20·0	20·5, 20·2
	50·0	50·2, 50·3
1 to 10	10·0	10·5, 10·5
	40·0	40·2, 40·2
	50·0	50·5, 50·2
1 to 100	10·0	10·3, 10·2
	20·0	20·2, 20·2

CONCLUSIONS

By using a paper chromatographic enrichment technique, it is possible to determine as little as 20 μg of vanadium as vanadate, in the presence of a 100-fold excess of chromium as dichromate, with an error of about 2 per cent.

Acknowledgment is made to the Department of Scientific and Industrial Research for Maintenance Grants (G.N. and A.J.B.).

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NOTE—Reference 11 constitutes Part I of this series.

DEPARTMENT OF INORGANIC AND PHYSICAL CHEMISTRY
THE UNIVERSITY
BRISTOL, 8

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A Modified Apparatus for the Quantitative Micro-determination of Carbon and Hydrogen in Organic Compounds

By F. E. CHARLTON

An existing combustion apparatus has been modified. The modified apparatus permits the satisfactory combustion of difficult substances more rapidly than is possible by using the Pregl apparatus. The value of manganese dioxide as an external absorbent for nitrogen oxides with a packed form of combustion tube and slow rates of flow (10 ml per minute) is confirmed. The advantages of the use of a third absorption tube have been extended. One common reason why high hydrogen figures may occur, and the manner in which this particular error may be eliminated, is given.

Most of the analyses carried out in this laboratory at one time involved compounds containing the elements phosphorus and fluorine, and with the standard Pregl[1] apparatus difficulty was encountered. No work was done on compounds containing C–F linkages. The compounds examined contained phosphorus directly linked to fluorine, *i.e.*, P–F linkages. With such compounds of unquestionable purity, the analytical figures obtained were neither satisfactory nor reproducible. By raising the main-heater and movable-heater temperatures to 700° C and 900° C, respectively, and maintaining the rate of flow of oxygen at 4 ml per minute, these difficulties were overcome, without the necessity of including additional reagents in the combustion train, but it was felt that it was necessary to shorten the time taken for an analysis. With this object in view an early Ingram[2] apparatus was modified. Technique too has been varied, resulting finally in the production of analytical figures as reliable as those obtained with the modified Pregl apparatus used in this laboratory, and it has been found possible to increase the number of analyses carried out daily.

Experimental

The preheater (A, Fig. 2), which operates at 340° C, and the heating mortar (D, Fig. 1), which operates at 250° C, were given separate controls, whereas previously they had been in series. The heating mortar was fitted with a Sunvic hot-wire vacuum switch (D′, Fig. 1) controlled by a mercury switch (E, Fig. 1). Chromel - Alumel thermocouples were installed with the main heater (B, Fig. 1), which operates at 700° C, and the movable heater (C, Fig. 1), which operates at 900° C. The thermocouples were connected through a two-way switch (F, Fig. 1) to a temperature recorder (G, Fig. 1). The main heater was lengthened and its position was adjusted so that the space between it and the heating mortar was reduced to approximately 5 mm. The object was to minimise the cooling of the combustion gases and so the possibility of condensation of water vapour in the side-arm of the water tube, a tendency noticed in the original apparatus. The slot beneath the movable heater was increased in length and the conducting leads were taken separately, to the ends of the heater, through this slot. Thus, movement of the heater was facilitated, its length of traverse was increased and it was more accessibible for repair. This last feature is the more desirable, since, because of its constant movement, the movable heater is the one most likely to fail.

The expensive combustion tube was replaced by a standard transparent silica tube conforming to the British Standard.[3] The side-arm was cut and connection was made by treated rubber tubing through the front panel via a horizontal slot to the scavenging train (Fig. 2, H and H′), which had been modified to make this possible. In this way the combustion tube had adequate support while being capable of easy adjustment horizontally and the possibility of breakage was minimised.

A packed filling was used instead of the Ingram boat filling. This was very similar to the standard Pregl filling. Rolls of 60-mesh silver gauze were used instead of silver-wool, a 4-cm roll taking the place of both the lead peroxide and the beak-end silver-wool filling. In addition to trapping undesirable gases that may escape the first silver gauze and the oxidising filling, this gauze prevents condensation of moisture[4] before the water-tube side-arm

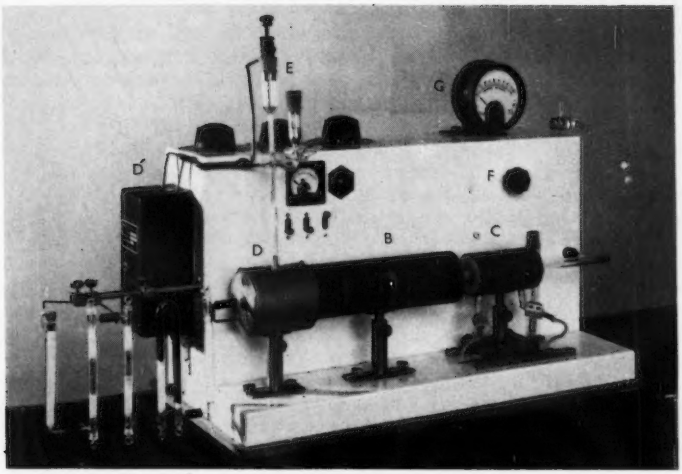


Fig. 1. Front of apparatus: B, main heater; C, movable heater; D, heating mortar; D′, Sunvic hot-wire vacuum switch; E, mercury switch; F, two-way switch; G, temperature recorder

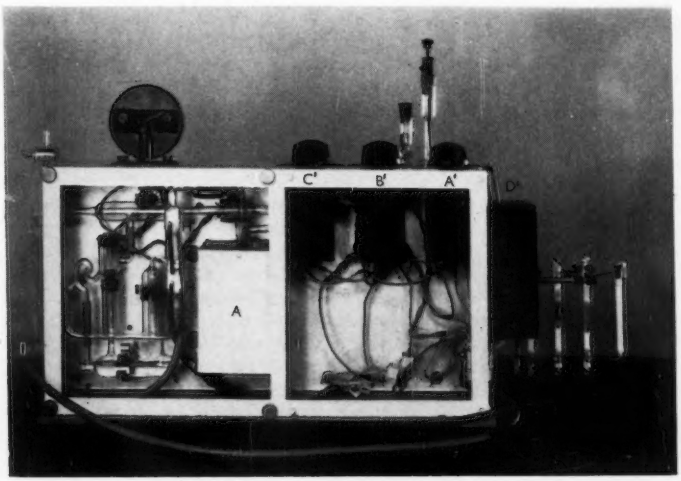


Fig. 2. Back of apparatus: A, preheater; D′, Sunvic hot-wire vacuum switch; H and H′, scavenging train; I, pinchcock

[To face p.582

is reached. To further this, the beak-end platinum wire is crimped into the gauze, the usual choking plug being omitted, and when the absorption train is connected a copper clip (visible in Fig. 1) conducts heat from the heating mortar to the water-tube side-arm.

Black granular manganese dioxide is used externally to retain oxides of nitrogen.[4] It is contained in a U-tube having ground-glass stoppers. The whole of one arm, and half of the other, is filled with manganese dioxide, the filling being completed with anhydrone. The reagents are held and separated by cotton-wool plugs. Originally, in accord with the idea then prevalent that the reagent must be pretreated before use, the manganese dioxide was dried in the U-tube before the addition of the anhydrone. Air, dried in passage through a bubbler containing 75 ml of concentrated sulphuric acid, was sucked through the manganese dioxide at 500 ml per minute for 60 minutes. The air was passed in the direction the oxygen would pass during a determination, and after 30 litres had passed the anhydrone was added to the U-tube. The carbon figures obtained from the first two determinations when the U-tube is included in the absorption train are slightly low, but thereafter are correct. Later, untreated maganese dioxide was used with precisely the same effect. The "Manganese Oxide Black (Dioxide), Granular for Batteries," that was used was supplied by The British Drug Houses Ltd. The U-tube is placed between the water and carbon dioxide tubes with the arm containing the anhydrone next to the carbon dioxide tube. This prevents moisture from the manganese dioxide being weighed as carbon dioxide. Twenty grams of manganese dioxide gave protection to the carbon dioxide tube from oxides of nitrogen for the analyses of about 150 compounds containing nitrogen.

Semi-micro absorption tubes of the Flaschenträger type are used. The filling of the water tube is anhydrone, but the filling of the carbon dioxide tube differs slightly from the usual in that the central two-thirds contain Carbosorb and both ends contain anhydrone. It is considered that this method of filling favours quicker and more permanent stabilising of conditions between the tubes. The use of a third tube[5] with the same filling as the carbon dioxide tube has certain advantages. Placed in the train during determinations, between the carbon dioxide and guard tubes, it is subject to the same conditions as the other two tubes, and is used as a counterpoise when weighing. The carbon dioxide tube becomes exhausted in less than one-half the time taken for the water tube. If, however, there is no appreciable stoppage, the counterpoise and carbon dioxide tubes may be interchanged. This applies equally well to Pregl-type tubes used with Pregl-type apparatus. The time is doubled betore the reagents must be replenished, and twice the number of determinations is possible with the same amount of material. Since all three tubes have the same surface area and are stoppered, temperature and humidity factors are eliminated. Determinations may be made in a room other than the balance room, which will then be unaffected by the combustion furnaces. Variation in balance zero, owing to temperature change, is reduced. The stoppered-tube fillings are always protected from the atmosphere, so no stabilising experiment is necessary, and with use of the last observed weights an extra analysis is possible daily.

USE OF THE APPARATUS—

Before making a determination, newly filled absorption tubes must be stabilised by the combustion of a micro-quantity of some easily combustible substance. The absorption train is connected, with all stoppers closed, to the combustion tube. The pinchcock (I, Fig. 2) is opened wide so that the rate of flow of oxygen is 40 to 50 ml per minute (predetermined by a pressure regulator not shown), when the bung is removed from the combustion tube. Previously the flowmeter gauge should have indicated no gas flow, since the system was closed. The boat containing the sample and the baffle are placed in the combustion tube as required and the bung is replaced. Because of the oxygen counterflow no atmospheric carbon dioxide or moisture can have entered the combustion tube. With volatile liquid samples, the boat when placed in the combustion tube must be pushed immediately beyond the side-arm, otherwise sample vapour may be swept into the atmosphere. When the bung is replaced, if there are no leaks in the system the flowmeter will read zero. The absorption train stoppers are opened and the rate of flow of oxygen is adjusted by the pinchcock to 10 ml per minute, the best rate of flow so far found for general purposes. With the movable heater in its first position the temperature is raised to 820° to 830° C in 10 minutes. It is now moved up to touch the main heater, bringing the sample centrally under it, and the temperature is raised to 900° to 910° C in the next 5 minutes. This temperature is maintained for a further 15 minutes, after which the movable heater is moved away from the main heater

and allowed to cool. The rate of flow of oxygen is reduced to 5 ml per minute, and the absorption train is disconnected after the stoppers have been closed, the one nearest the beak first, the one nearest the guard tube next, and subsequently the others. In the course of wiping and weighing, the carbon dioxide tube is wiped last, as it is weighed last.

The positions occupied by the sample boat and the movable heater depend on the nature of the sample, as follows—

Sample boat—The distances it is placed from the silver gauze found to be most satisfactory are: high-melting solids, 1 cm; low-melting solids and high-boiling liquids, 1·5 cm; low-boiling liquids, 2 cm.

First position of the movable heater—High-melting solids, just covering the boat; low-melting solids and high-boiling liquids, just at the end of the boat handle; low-boiling liquids, 1 cm from the boat handle.

Second position of the movable heater—With all samples, touching the main heater.

For no apparent reason some analysts still seem to get high hydrogen results with errors of variable magnitude. This is because sample boats and liquid sampling tubes always have a film of moisture on them, which varies according to the atmospheric humidity. Since this film is always present, variation in the hydrogen figures obtained may result, although the carbon figures obtained may be correct and remain unaltered. The error in the hydrogen figure may be eliminated with solids by weighing the sample just before removing the absorption tubes from the previous experiment and standing the boat containing the sample in a desiccator until required. With liquids the same procedure is adopted for the boat that is to contain the opened sample tube, and the sample tube just before cutting open is wiped with a dry chamois leather. Sampling apparatus, other than boats, is kept in desiccators until required, and hygroscopic samples are taken in a "dry-box."

ADVANTAGES OF THE MODIFIED APPARATUS OVER THE PREGL APPARATUS—

(*a*) Manganese dioxide granules are used, without treatment, from stock.

(*b*) Silver gauzes are easily made and are easy to purify for re-use.

(*c*) The combustion tube is quickly and easily filled without fear of blockage.

(*d*) The combustion tube requires no initial conditioning period. When the heaters reach operating temperatures, it is ready for use.

(*e*) No daily stabilising experiment is required. An extra determination can be made daily.

(*f*) Flaschenträger absorption tubes are easily cleaned and filled. The side-arm bore is not critical and a wide variation in the rate of flow of oxygen is possible. The stoppered tubes exclude moisture and carbon dioxide from the atmosphere and so may be reconnected whenever convenient.

(*g*) The apparatus and balances may occupy rooms with different temperatures and humidities.

(*h*) Twenty minutes are saved on each determination.

(*i*) A ruined determination as a result of explosive combustion is unusual because of the baffle and anti-blowback device.

(*j*) The apparatus is very compact.

(*k*) With the automatic temperature recorder, and individual heater control, adjustment is simple.

(*l*) Control of the rate of flow of oxygen is simple and immediate.

RESULTS

Some of the compounds analysed by using this modified apparatus are listed. Check analyses were made repeatedly, with salicylic acid for ordinary compounds, acetanilide for nitrogen compounds and triphenyl phosphate for phosphorus compounds. The average figures found for the analyses are shown in Table I.

TABLE I

ANALYSES OF VARIOUS COMPOUNDS

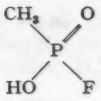
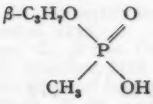
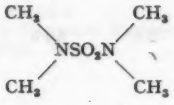
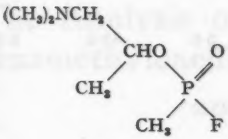
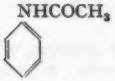
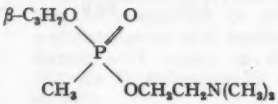
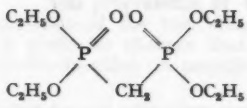
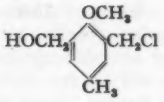
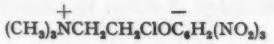
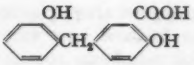
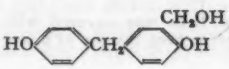
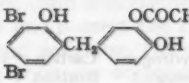
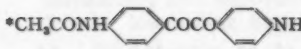
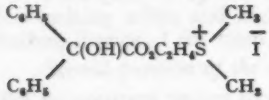
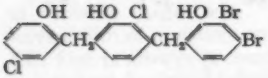
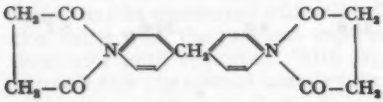
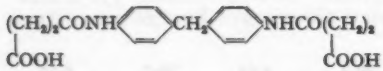
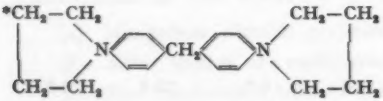
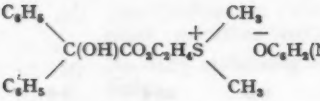
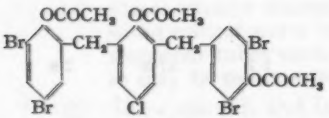
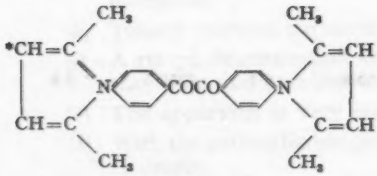
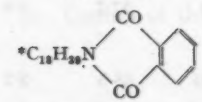
Compound	Atomic formula	Carbon content calculated, %	Hydrogen content calculated, %	Carbon content found, %	Hydrogen content found, %
	CH_4O_2FP	12·25	4·1	12·0	4·1
	$C_4H_{11}O_3P$	34·8	8·0	34·7	8·1
	$C_4H_{12}O_2N_2S$	31·6	8·0	31·6	8·2
	$C_6H_{15}O_2NFP$	39·3	8·25	39·5	8·55
	C_8H_9ON	71·1	6·7	71·2	6·9
	$C_8H_{20}O_3NP$	45·9	9·6	46·1	9·8
	$C_8H_{22}O_6P_2$	37·5	7·7	37·3	7·9
	$C_{10}H_{13}O_2Cl$	59·8	6·5	59·5	6·6
	$C_{11}H_{13}O_7ClN_4$	37·7	4·3	37·7	4·6
	$C_{14}H_{12}O_4$	68·8	4·95	68·6	5·2
	$C_{14}H_{14}O_3$	73·0	6·1	72·7	6·3

TABLE I—*continued*

	$C_{15}H_{12}O_4Br_2$	43·3	2·9	43·5	3·1
$(C_6H_5O)_2P{=}O$	$C_{18}H_{15}O_4P$	66·3	4·6	66·4	4·8
	$C_{18}H_{16}O_4N_2$	66·7	5·0	66·5	5·2
	$C_{18}H_{21}O_2IS$	48·65	4·8	48·85	4·95
	$C_{20}H_{14}O_3Cl_2Br_2$	45·1	2·65	45·3	2·85
	$C_{21}H_{18}O_4N_2$	69·6	5·0	69·5	5·1
	$C_{21}H_{22}O_6N_2$	63·3	5·6	63·6	5·4
	$C_{21}H_{26}N_2$	82·3	8·6	82·6	8·9
	$C_{24}H_{22}O_{10}N_3S$	52·8	4·25	52·9	4·4
	$C_{26}H_{19}O_6ClBr_4$	39·9	2·45	40·1	2·7
	$C_{26}H_{26}O_2N_2$	78·8	6·1	78·9	6·4
	$C_{26}H_{41}O_2N$	78·1	10·3	78·4	10·6

* The sample immediately before introduction into the combustion tube was covered with dry, powdered, fused potassium dichromate to assist in the breakdown of any refractory residue of nitrogenous charcoal that might possibly form.

Scope and validity of the results

Reference to the formulae of the compounds listed in Table I indicates that some rather unusual types of compound were analysed by using this apparatus. This indeed is why a departure from normal practice has been made and structural formulae have been included. With many compounds elements other than carbon and hydrogen were determined, and physical checks, including infra-red measurements, were made on the compounds analysed, but inclusion of these details in this paper would render it cumbersome. The modified apparatus is of use in research work involving unusual types of compound.

I thank Mr. G. Ingram, with whom I discussed this paper, and also the Chief Scientist, Ministry of Supply, for permission to publish. The photographs are reproduced by permission of the Controller, H.M. Stationery Office.

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Ministry of Supply
Chemical Defence Experimental Establishment
Porton, Wilts.

March 5th, 1956

The Analysis of Mixtures of the Hydrochlorides of Hexamethylenediamine, *p*-Diaminodi*cyclo*hexylmethane and ϵ-Aminocaproic Acid

By M. Clasper, J. Haslam and E. F. Mooney

The method developed for the chromatographic separation and determination of mixtures of the hydrochlorides of hexamethylenediamine, *p*-diaminodi*cyclo*hexylmethane and ϵ-aminocaproic acid involves the separation of a mixture of *p*-diaminodi*cyclo*hexylmethane dihydrochloride and ϵ-aminocaproic acid hydrochloride on a cellulose column, with *sec.*-butanol - formic acid - water as eluting solvent. Hexamethylenediamine dihydrochloride is subsequently eluted from the column with an alcohol - water solvent.

The proportions of the respective hydrochlorides are determined by analysis of the two eluates after evaporation to dryness, and allowance is made for changes that take place in the composition of the individual hydrochlorides on passage through the column.

In a previous paper[1] attention was drawn to the chromatographic separation of the hydrochlorides of ϵ-aminocaproic acid, hexamethylenediamine and *p*-diaminodi*cyclo*hexylmethane. Experiments on cellulose columns had indicated that it was possible to separate hexamethylenediamine dihydrochloride from the other two hydrochlorides, but no quantitative work had been carried out on the system. It seemed desirable to carry out such work, because if successful it would permit satisfactory conclusions to be drawn about the composition of interpolymers of nylon 6, nylon 66 and nylon PACM 6.

Experimental

Preliminary tests were carried out, as a result of which a procedure based on the following considerations was worked out.

An aqueous alcoholic solution of the three hydrochlorides was applied to a cellulose column and then eluted with a solvent mixture containing definite proportions of *sec.*-butanol, formic acid and water. After passage of a known volume of this solvent through the column, the eluate was evaporated to dryness, and a determination of total acidity and total chlorine

was carried out on the residue obtained. From these determinations the proportions of ϵ-aminocaproic acid hydrochloride and *p*-diaminodi*cyclo*hexylmethane dihydrochloride in this eluate were calculated. The hexamethylenediamine dihydrochloride in the column was then eluted with an alcohol - water solvent of definite composition and the eluate was subsequently evaporated to dryness. Determination of chloride in this residue gave a measure of the hexamethylenediamine dihydrochloride in this portion of the eluate.

When this test was applied to mixtures of (*a*) ϵ-aminocaproic acid hydrochloride and hexamethylenediamine dihydrochloride, (*b*) *p*-diaminodi*cyclo*hexylmethane dihydrochloride and hexamethylenediamine dihydrochloride, and (*c*) ϵ-aminocaproic acid hydrochloride, *p*-diaminodi*cyclo*hexylmethane dihydrochloride and hexamethylenediamine dihydrochloride, the results shown in Table I were obtained.

Table I

Chromatographic separation of mixtures of the base hydrochlorides, followed by their volumetric determination

	Mixture of ϵ-aminocaproic acid hydrochloride and hexamethylenediamine dihydrochloride		Mixture of *p*-diaminodi*cyclo*hexylmethane dihydrochloride and hexamethylenediamine dihydrochloride		Mixture of ϵ-aminocaproic acid hydrochloride, *p*-diaminodi*cyclo*hexylmethane dihydrochloride and hexamethylenediamine dihydrochloride	
	Added, g	Found, g	Added, g	Found, g	Added, g	Found, g
ϵ-Aminocaproic acid hydrochloride	0·0928	0·0878	—	—	0·1012	0·0963
p-Diaminodi*cyclo*hexylmethane dihydrochloride	—	—	0·1000	0·0973	0·0516	0·0497
Hexamethylenediamine dihydrochloride	0·1063	0·0974	0·1000	0·0965	0·0499	0·0480

It will be seen that all the recoveries were low, but in general the recovered substances are present in proportions that closely approximate to those in the original mixtures.

In a second set of experiments on two component mixtures of known composition the material recovered in each fraction from the column was weighed. This material was then redissolved and titrated by the procedure outlined above. The results obtained are shown in Table II.

Table II

Chromatographic separation of mixtures of the base hydrochlorides, followed by their gravimetric and volumetric determination

	Mixture of ϵ-aminocaproic acid hydrochloride and hexamethylenediamine dihydrochloride			Mixture of *p*-diaminodi*cyclo*hexylmethane dihydrochloride and hexamethylenediamine dihydrochloride		
	Added, g	Found by weight, g	Found by titration, g	Added, g	Found by weight, g	Found by titration, g
ϵ-Aminocaproic acid hydrochloride	0·0488	0·0496	0·0450	—	—	—
p-Diaminodi*cyclo*hexylmethane dihydrochloride	—	—	—	0·0938	0·0904	0·0900
Hexamethylenediamine dihydrochloride	0·1489	0·1456	0·1357	0·1060	0·1079	0·0999

It will be noted from these results that the amounts found by weighing the evaporated eluates were in quite good agreement with the amounts of the respective hydrochlorides added. Nevertheless, the figures obtained by titration of the evaporated eluates did not agree with their weights, *i.e.*, there was evidence that some change had taken place in the composition of the respective hydrochlorides either on the column or on evaporation of the eluates.

In order to examine this point in greater detail, experiments were carried out on hexamethylenediamine dihydrochloride alone.

A known weight of the material was submitted to the chromatographic test, with the results shown in Table III.

Table III

Gravimetric and volumetric determination of hexamethylenediamine dihydrochloride after passing through a chromatographic column

	Hexamethylenediamine dihydrochloride		
	Added, g	Found by weight, g	Found by titration, g
Column 1	0·2084	0·2047	0·1969
Column 2	0·2105	0·2075	0·2010
Column 3	0·2108	0·2087	0·2025
Column 4	0·2024	0·1999	0·1943

The infra-red spectrum of the material recovered from the eluate was then compared with the spectrum of the original hexamethylenediamine dihydrochloride. These spectra are given in Fig. 1.

Although the spectra are very similar, the material from the eluate shows increased absorption at about 1620 cm^{-1}, which is evidence of the presence of a constituent containing a carbonyl grouping.

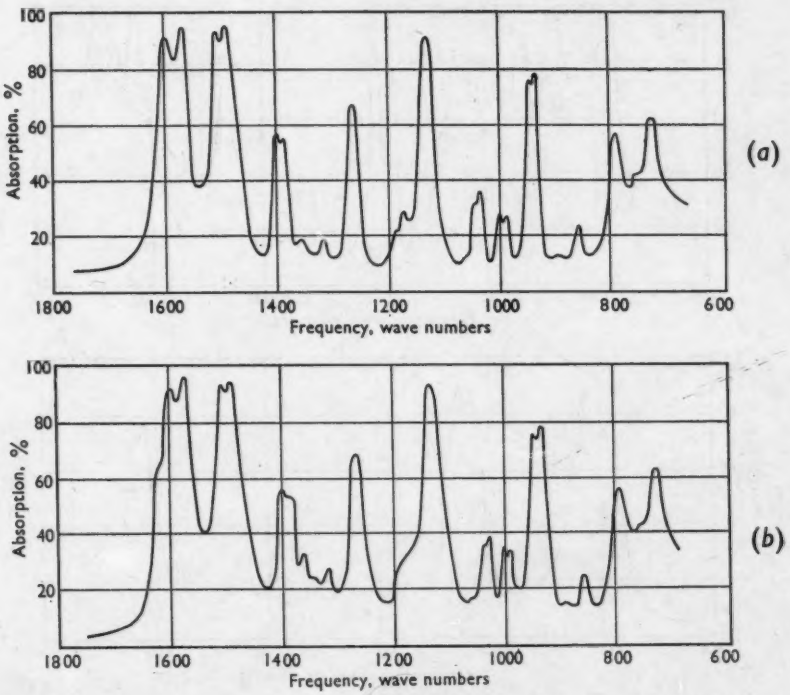


Fig. 1. Infra-red spectra of: (*a*) hexamethylenediamine dihydrochloride; (*b*) hexamethylenediamine dihydrochloride that has been eluted from the chromatographic column. The spectra were prepared by the potassium bromide disc method; 1 per cent. of substance in potassium bromide; thickness of disc 1 mm

Since formic acid is used in the eluting solvent, it was suspected at this stage that the material from the column might be contaminated with either the diformyl or the dihydroformate derivative of hexamethylenediamine.

These derivatives were prepared by the following procedures—

Hexamethylenediamine diformyl—Hexamethylenediamine was heated under reflux with 90 per cent. formic acid and then taken to dryness; the residue was recrystallised from ethanol. This material was found to have the following composition on analysis—

Carbon = 55·9 per cent. (theoretical value 55·7 per cent.)
Hydrogen = 9·2 per cent. (theoretical value 9·4 per cent.)
Oxygen = 18·8 per cent. (theoretical value 18·6 per cent.)
Nitrogen = 16·6 per cent. (theoretical value 16·3 per cent.)

The infra-red spectrum of this material is shown in Fig. 2.

Hexamethylenediamine dihydroformate—Hexamethylenediamine was neutralised with 90 per cent. formic acid in ethanol solution. Acetone was added to the solution and the precipitate obtained was filtered off and washed with acetone. This material was found to have the following composition on analysis—

Carbon = 45·9 per cent. (theoretical value 46·0 per cent.)
Hydrogen = 9·9 per cent. (theoretical value 9·7 per cent.)
Oxygen = 31·1 per cent. (theoretical value 30·7 per cent.)
Nitrogen = 13·5 per cent. (theoretical value 13·5 per cent.)

The infra-red spectrum of this material is shown in Fig. 2.

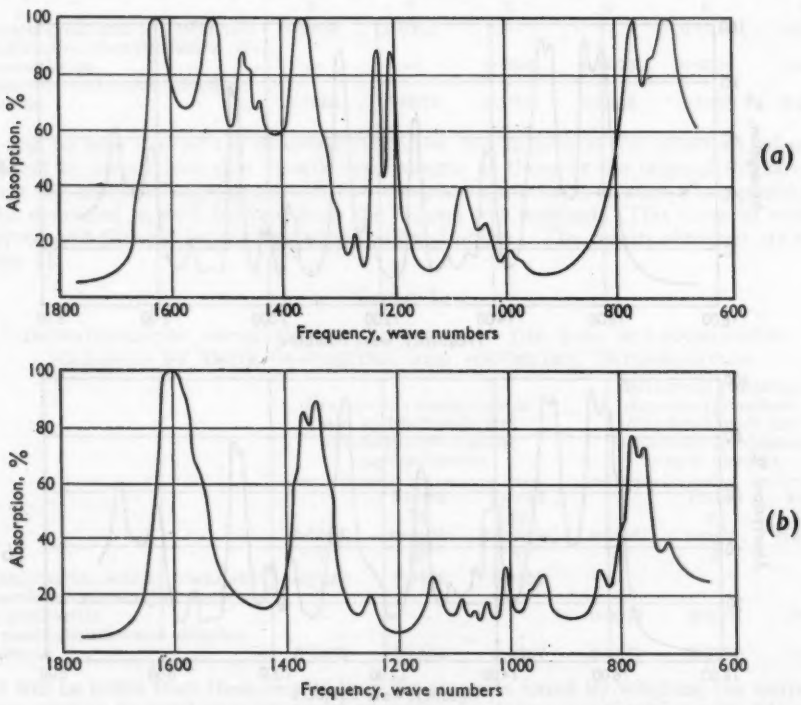


Fig. 2. Infra-red spectra of: (*a*) the diformyl derivative of hexamethylenediamine; (*b*) hexamethylenediamine dihydroformate. The spectra were prepared by the potassium bromide disc method; 1 per cent. of substance in potassium bromide; thickness of disc 1 mm

Mixtures of hexamethylenediamine dihydrochloride with (*a*) 5 per cent. by weight of the diformyl derivative, and (*b*) 5 per cent. by weight of the dihydroformate were now prepared, and their infra-red spectra were recorded. These spectra are shown in Fig. 3. Examination of these spectra did not point to the definite presence of either the diformyl or dihydroformate derivative of the hexamethylenediamine in the substance recovered from the chromatographic column.

Direct determination of the oxygen content by the Unterzaucher method, however, on the hexamethylenediamine dihydrochloride before and after passage through the chromatographic column yielded strong evidence that the material recovered from the eluate included an oxygen-containing constituent, as shown by the results in Table IV.

TABLE IV

OXYGEN DETERMINATIONS ON HEXAMETHYLENEDIAMINE DIHYDROCHLORIDE BEFORE AND AFTER PASSING THROUGH A CHROMATOGRAPHIC COLUMN

	Weight taken, mg	Titre of 0·2 *N* sodium thiosulphate, ml	Oxygen content, %
Hexamethylenediamine dihydrochloride	51·95	0·63	0·16
	51·18	0·89	0·23
Hexamethylenediamine dihydrochloride recovered from chromatographic column	51·88	4·31	1·11
	52·55	4·67	1·19
Blank on apparatus	—	0·24	0·06*
	—	0·21	0·05*

* These results are calculated on a weight of 52·0 mg.

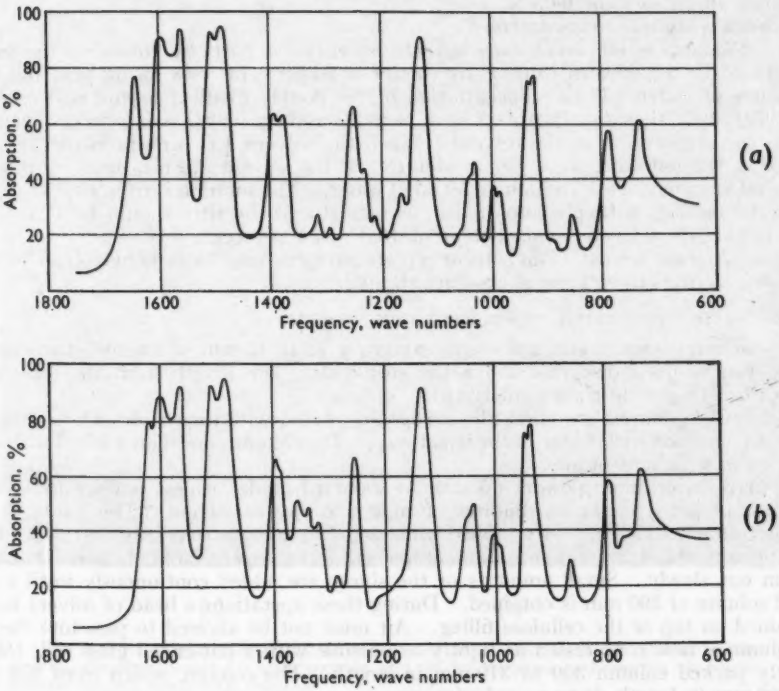


Fig. 3. Infra-red spectra of: (*a*) hexamethylenediamine dihydrochloride containing 5 per cent. of diformyl derivative; (*b*) hexamethylenediamine dihydrochloride containing 5 per cent. of dihydroformate. The spectra were prepared by the potassium bromide disc method; 1 per cent. of substance in potassium bromide; thickness of disc 1 mm

Evidence that the two substances were quite different was also provided by the application of the paper-chromatographic test described previously.[1]

Fig. 4 shows the paper chromatograms of the hexamethylenediamine dihydrochloride before and after passing through the chromatographic columns, as seen under ultra-violet light. Whereas hexamethylenediamine dihydrochloride shows one definite spot, there is evidence of a further two spots in the hexamethylenediamine dihydrochloride that has been eluted from the column.

Although we have been unable to identify the contaminant produced as a result of the chromatographic test on hexamethylenediamine dihydrochloride, we believe that the following method would give a satisfactory resolution of mixtures of the three hydrochlorides of ϵ-aminocaproic acid, *p*-diaminodi*cyclo*hexylmethane and hexamethylenediamine.

The method is based on the assumption that the three hydrochlorides calculated as shown in the test are in the same proportions as in the mixture of hydrochlorides submitted to the test.

Method

Reagents—

sec.-*Butanol, redistilled.*

Formic acid, doubly distilled—The concentration of the doubly distilled acid is determined in the following manner. Approximately 2 ml of the acid are weighed into a stoppered weighing bottle, and the bottle is transferred to a 250-ml flask containing 100 ml of distilled water. The stopper is removed from the bottle under water and the solution is titrated with *N* sodium hydroxide solution to the end-point with phenolphthalein.

1 ml of *N* sodium hydroxide ≡ 0·04603 g of formic acid.

Ethanol, absolute.

Silver nitrate solution, 0·05 N.

Sodium hydroxide solution, 0·05 N.

sec.-*Butanol - formic acid - water solvent*—Seventy-five parts by volume of the redistilled *sec.*-butanol are mixed with 15 parts by volume of 90 per cent. w/w formic acid and 10 parts by volume of water. If the concentration of the doubly distilled formic acid differs from 90 per cent. w/w, then an adjustment must be made in the proportion of formic acid and water used in the preparation of the solvent. When the solvent has been prepared, it is finally checked in the following way. Five millilitres of the solvent are measured by means of a pipette into a conical flask containing 50 ml of water. The solution is titrated with *N* sodium hydroxide solution, with phenolphthalein as indicator. The titre should be 17·2 ± 0·1 ml, which represents a formic acid content of 15·8 ± 0·1 per cent. w/v.

Ethanol - water solvent—This solvent is prepared by mixing 75 parts by volume of distilled water and 25 parts by volume of absolute ethanol.

Procedure for preparing chromatographic columns—

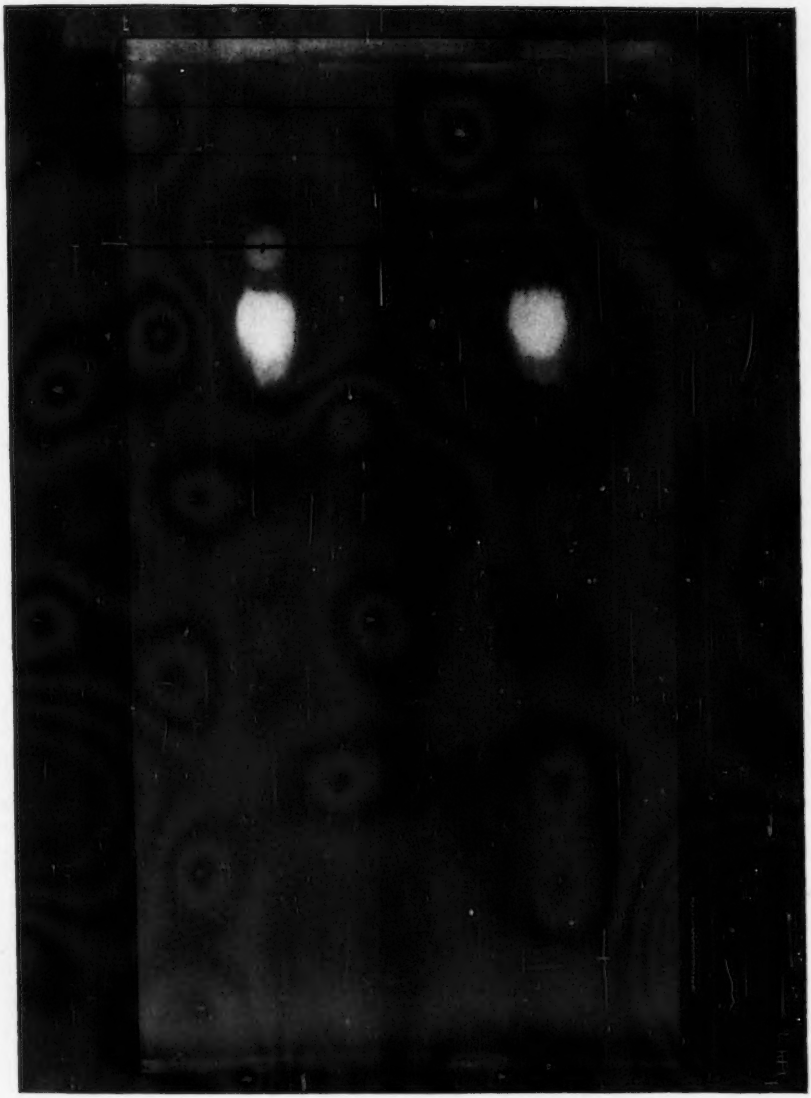
The columns used are of glass, approximately 12 to 13 mm in internal diameter, fitted with a sintered-glass disc just above the stop-cock. The length from the sintered-glass disc to the B14 ground-glass joint is 440 to 450 mm.

Before the columns are filled, the insides are coated with dimethyldichlorosilane, which is then decomposed with water in the usual way. The columns are then washed with acetone and dried in a current of air.

A slurry of cellulose powder (Whatman standard grade, ashless powder for chromatography) is prepared in the *sec.*-butanol - formic acid - water solvent. This slurry is poured into the column, which is gently tapped while doing so in order to ensure that no air bubbles are trapped in the slurry. The column stop-cock is then opened and the solvent is allowed to drain out slowly. Small amounts of the slurry are added continuously until a loosely packed column of 390 mm is obtained. During these operations a head of solvent is always maintained on top of the cellulose filling. Air must not be allowed to pass into the filling. The column is now compressed as tightly as possible with a flat-ended glass rod; this gives a tightly packed column 300 to 310 mm in length. The column, which must not be less than 300 mm in length, is now ready for use.

Procedure for chromatographic separation—

A 0·2-g portion of the mixed hydrochlorides is weighed into a 10-ml beaker; 0·2 ml of distilled water and 0·3 ml of the doubly distilled formic acid are added, and the beaker is



(*a*) (*b*)

Fig. 4. Paper chromatograms, as seen under ultra-violet light, of hexamethylenediamine dihydrochloride (*a*) after passing and (*b*) before passing through a chromatographic column filled with cellulose

[To face p.592

swirled occasionally until solution is attained. When the hydrochlorides have dissolved, 1·5 ml of *sec.*-butanol are added. The solution is now transferred to the top of the column by pouring through a small funnel with a drawn out stem. The beaker is washed three times with 1-ml portions of the *sec.*-butanol - formic acid - water solvent and finally the funnel is washed round with 1 ml of the solvent.

One hundred millilitres of the *sec.*-butanol - formic acid - water solution are placed in the solvent reservoir, which consists of a 500-ml round-bottomed flask with the neck drawn out to form a stem of ¼ inch in diameter. When the solvent washings have drained from the top of the column, the reservoir is placed on the top of the column so that the bottom of the stem is about 100 mm above the top of the cellulose packing. The solvent is allowed to flow slowly through the column and the eluate is collected in a 250-ml beaker.

When the *sec.*-butanol - formic acid - water solvent has just drained from the top of the column, the empty reservoir is replaced with one containing 100 ml of the ethanol - water solvent. The 250-ml beaker is replaced by a second clean one, and the solvent is allowed to drain through the column.

The first fraction, *i.e.*, the *sec.*-butanol - formic acid - water solution, contains ε-aminocaproic acid hydrochloride and *p*-diaminodi*cyclo*hexylmethane dihydrochloride, and the second fraction, *i.e.*, the ethanol - water solution, contains hexamethylenediamine dihydrochloride.

PROCEDURE FOR DETERMINING BASE HYDROCHLORIDES IN FRACTIONS—

After 20 ml of distilled water have been added to each 250-ml beaker, the solvent is removed by placing the beaker on a water bath. The residues are then dissolved in a small amount of water and re-evaporated. Evaporation with small amounts of water is repeated three or four times. Fifteen millilitres of water are then added and, after the beaker has been warmed on the water bath to ensure that complete solution has been obtained, the solution is allowed to cool.

The first fraction (*sec.*-butanol - formic acid - water fraction) is titrated with 0·05 *N* sodium hydroxide solution, with phenolphthalein as indicator. Then 1·5 ml of 2 *N* nitric acid are added, and the chloride ion is titrated electrometrically with 0·05 *N* silver nitrate solution. From the 0·05 *N* sodium hydroxide titre obtained, the weight of ε-aminocaproic acid hydrochloride is calculated, and from the difference between the 0·05 *N* silver nitrate titre and the 0·05 *N* sodium hydroxide titre, the *p*-diaminodi*cyclo*hexylmethane dihydrochloride is calculated.

To the second fraction are added 1·5 ml of 2 *N* nitric acid solution, and the chloride ion is titrated electrometrically with 0·05 *N* silver nitrate solution. From the titre obtained, the hexamethylenediamine dihydrochloride content of the mixed sample is calculated.

A blank determination is carried out.

CALCULATION OF RESULTS

The weight of each hydrochloride obtained in the test is divided by the total weight of hydrochlorides obtained and is then multiplied by 100 to give the percentage of hydrochloride in the original mixture, for example—

Weight of sample taken	0·20 g
Weight of ε-aminocaproic acid hydrochloride found	0·09 g
Weight of *p*-diaminodi*cyclo*hexylmethane dihydrochloride found	0·06 g

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Replace the 4th line from the foot of the page by—

$$\text{Hexamethylenediamine dihydrochloride in sample} = \frac{0{\cdot}03}{0{\cdot}18} \times 100 = 16{\cdot}7 \text{ per cent.}$$

ε-Aminocaproic acid dihydrochloride in sample = $\frac{\quad}{0{\cdot}18}$

RESULTS

Application of the method described to various mixtures of the three hydrochlorides gave the results shown in Table V.

TABLE V

ANALYSIS OF KNOWN MIXTURES OF THE BASE HYDROCHLORIDE

Composition of mixtures					
ε-Aminocaproic acid hydrochloride		*p*-Diaminodi*cyclo*hexylmethane dihydrochloride		Hexamethylenediamine dihydrochloride	
Added, %	Found, %	Added, %	Found, %	Added, %	Found, %
30	29	—	—	70	71
89	89	—	—	11	11
50	50	25	25	25	25
10	8	15	16	75	76
—*	1	50	50	50	49
74*	73	12	13	14	14

* The composition of these mixtures was unknown to the operator at the time of the test.

We are indebted to Mr. H. A. Willis for the infra-red spectra shown in this paper.

REFERENCE

1. Clasper, M., Haslam, J., and Mooney, E. F., *Analyst*, 1955, **80**, 812.

IMPERIAL CHEMICAL INDUSTRIES LIMITED
PLASTICS DIVISION
WELWYN GARDEN CITY, HERTS.

March 28th, 1956

The Determination of Epichlorhydrin in Air

BY J. W. DANIEL AND J. C. GAGE

A sensitive colorimetric method is described for the determination of epichlorhydrin in the atmosphere. It is based on oxidation with periodic acid, followed by reaction of the formaldehyde produced with ammonia and acetylacetone to give a yellow colour. The method has been checked on atmospheres containing epichlorhydrin in known concentrations.

EPICHLORHYDRIN has found useful applications in synthetic organic chemistry owing to the reactivity of its epoxide ring. Its vapour is toxic; no reliable experiments appear to have been made to determine a maximum allowable concentration, but work at present in progress in these laboratories suggests that the limit for prolonged exposure should not be greater than 20 mg per cubic metre.

Epichlorhydrin may be determined in aqueous solution by the well known reaction with sodium salts of suitable anions, in which alkali is liberated, which can then be titrated with acid. This procedure is, however, of low specificity and sensitivity, and at atmospheric concentrations less than 20 mg per cubic metre would require the collection of a large air sample. In the present method the epichlorhydrin is oxidised with periodic acid to formaldehyde, which is then determined colorimetrically by a sensitive reaction.

EXPERIMENTAL

DETERMINATION OF FORMALDEHYDE—

The simplest and most suitable method for the determination of formaldehyde was found to be that of Nash,[1] in which formaldehyde reacts with ammonia and acetylacetone to form 3:5-diacetyl-1:4-dihydrolutidine, which has a yellow colour. Maximum colour development occurs at a final pH value of about 6·0; this is achieved by means of an ammonium acetate - acetic acid buffer. The full colour was found to require 2 hours to develop at room temperature but only 2 minutes at 100° C.

OXIDATION WITH PERIODIC ACID—

When an aqueous solution of periodic acid was subjected to the procedure for formaldehyde determination, a yellow colour developed. When the periodic acid was destroyed with sodium

arsenite, this interference was prevented; other reducing agents either did not prevent the yellow colour from periodic acid or inhibited the subsequent colour reaction for formaldehyde.

The effect of experimental conditions on the oxidation of epichlorhydrin to formaldehyde is shown graphically in Fig. 1. An aqueous solution containing 50 μg of epichlorhydrin in 10 ml was subjected to various treatments before and after the addition of 1 ml of 0·1 *M* periodic acid, after which the colorimetric procedure was completed as described below. The measured optical densities were referred to a standard curve constructed from the

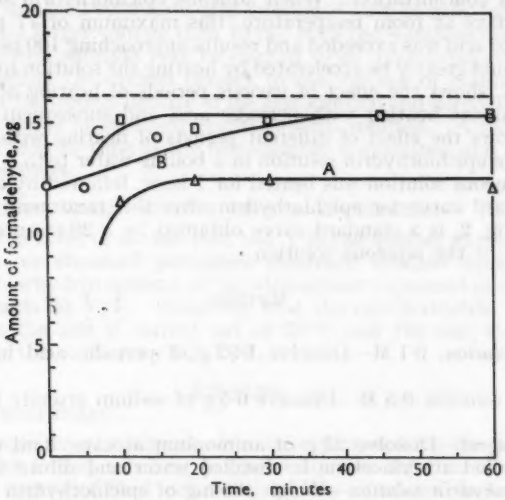

Fig. 1. Liberation of formaldehyde from epichlorhydrin: curve A, duration of heating with periodic acid, no previous heating with water; curve B, duration of heating with water, followed by a 30-minute heating with periodic acid; curve C, duration of heating with periodic acid after a 1-hour heating with water

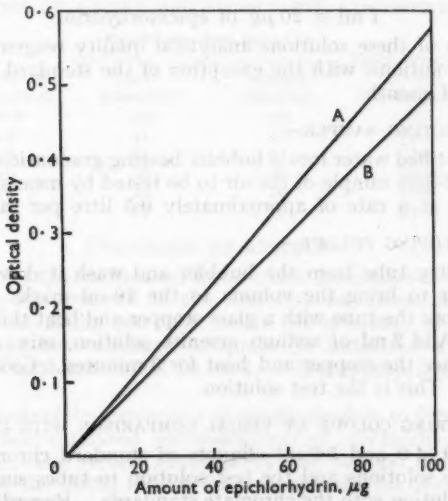

Fig. 2. Standard curves for epichlorhydrin: curve A, with preliminary heating in aqueous solution; curve B, without preliminary heating in aqueous solution

optical densities of a series of formaldehyde solutions submitted to the colour-development procedure in the presence of periodic acid and sodium arsenite; this enabled the amount of formaldehyde liberated to be calculated. It was found that when periodic acid acted at room temperature (25° C), formaldehyde was liberated very slowly. When the oxidation was effected at the temperature of a boiling-water bath, the formaldehyde measured reached a maximum within 30 minutes, which corresponded to 77 per cent. of the expected value (curve A, Fig. 1), and this maximum conversion could not be increased by a tenfold increase in the periodic acid concentration. When aqueous epichlorhydrin solutions were allowed to age for several days at room temperature, this maximum of 77 per cent. achieved by heating with periodic acid was exceeded and results approaching 100 per cent. were obtained. This ageing effect could greatly be accelerated by heating the solution in a boiling-water bath, and curve B, Fig. 1, shows the effect of various periods of heating of an aqueous solution, followed by a 30-minute heating with periodic acid and subsequent colour development. Curve C, Fig. 1, shows the effect of different periods of heating with periodic acid after a 1-hour heating of the epichlorhydrin solution in a boiling-water bath. For maximum colour development the aqueous solution was heated for 1 hour, followed by a 20-minute oxidation period, and a standard curve for epichlorhydrin after this treatment is shown in curve A, Fig. 2. Curve B, Fig. 2, is a standard curve obtained by a 20-minute oxidation without a preliminary heating of the aqueous solution.

Method

Reagents—

Periodic acid solution, 0·1 M—Dissolve 1·92 g of periodic acid in distilled water and dilute to 100 ml.

Sodium arsenite solution, 0·5 M—Dissolve 6·5 g of sodium arsenite in distilled water and dilute to 100 ml.

Acetylacetone reagent—Dissolve 25 g of ammonium acetate, 3 ml of glacial acetic acid and 0·2 ml of redistilled acetylacetone in distilled water and dilute to 100 ml.

Standard epichlorhydrin solution—Weigh 100 mg of epichlorhydrin and dilute to 100 ml with distilled water; dilute this solution 1 to 50 with distilled water. This solution should be freshly prepared.

1 ml of standard solution $\equiv$ 20 μg of epichlorhydrin.

Standard potassium chromate solution—Dissolve 100 mg of potassium chromate in water and dilute to 100 ml.

1 ml $\equiv$ 20 μg of epichlorhydrin.

In the preparation of these solutions analytical quality reagents should be used when available. All of the solutions, with the exception of the standard epichlorhydrin solution, are stable for at least 1 month.

Procedure for collecting sample—

Measure 8 ml of distilled water into a bubbler bearing graduation marks at 10 and 15 ml, and draw through it a 2-litre sample of the air to be tested by means of an aspirator or other suitable suction device at a rate of approximately 0·5 litre per minute.

Procedure for developing colour—

Remove the air-entry tube from the bubbler and wash it down, inside and out, with sufficient distilled water to bring the volume to the 10-ml mark. Add 1 ml of periodic acid solution, loosely close the tube with a glass stopper and heat the tube in a boiling-water bath for 20 minutes. Add 2 ml of sodium arsenite solution, mix, and add 2 ml of acetylacetone reagent. Replace the stopper and heat for 3 minutes. Cool and adjust the volume to 15 ml, if necessary. This is the test solution.

Procedure for measuring colour by visual comparison with chromate standards—

Dilute 1·0, 2·0, 3·0, 4·0 and 5·0-ml aliquots of standard chromate solution to 15 ml. Transfer these reference solutions and the test solution to tubes similar in size and colour, and compare the test solution with the chromate standards. Record the volume of standard chromate solution that gives a colour nearest to that of the test solution. To obtain a closer match intermediate chromate dilutions may be made.

PROCEDURE FOR INSTRUMENTAL COLOUR MEASUREMENT—

To a series of glass-stoppered tubes graduated at 10 and 15 ml, add 0, 1·0, 2·0, 3·0, 4·0 and 5·0-ml aliquots of standard epichlorhydrin solution. Adjust the volume to 10 ml with distilled water and develop the colour as described above for the test solution. Measure the optical densities of these solutions in a suitable photo-electric colorimeter at a wavelength band in the region of 412 mμ, selected by means of a monochromator or an Ilford No. 601 filter, and in cells with an optical depth of 1 cm. For cells with a greater optical depth smaller volumes of the standard epichlorhydrin solution should be used to prepare this series. If the cell used requires a larger volume than 15 ml, both the solutions used to construct the standard curve and the test solution may be diluted to an appropriate volume. Construct a calibration graph relating the optical density to the volume of standard epichlorhydrin solution used. Measure the optical density of the test solution and derive from the calibration graph the equivalent volume of standard epichlorhydrin solution. A reagent blank should also be determined at the same time as the test solution is examined and an allowance made for this in the usual manner.

CALCULATION OF EPICHLORHYDRIN CONTENT OF THE ATMOSPHERE—

If V is the volume in litres of air sampled and Y is the number of millilitres of standard epichlorhydrin solution or standard potassium chromate solution equivalent to the test solution, then the epichlorhydrin content of the atmosphere expressed in mg per cubic metre is given by the expression $20 \cdot Y/V$. Assuming that the epichlorhydrin vapour behaves as a perfect gas and that the test is carried out at 20° C and 760 mm, the concentration in p.p.m. v/v is given by the expression $5 \cdot 2\ Y/V$.

RESULTS

ANALYSIS OF TEST ATMOSPHERES—

Known test atmospheres of epichlorhydrin were prepared by the procedure described by Diggle and Gage[2] for keten; a freshly prepared aqueous solution of epichlorhydrin was injected by means of a controlled fluid feed atomiser[3] into a metered stream of air. A sample of the test atmosphere was drawn through two bubblers in series, each containing 8 ml of water, and these solutions were analysed by the procedure described above. The results obtained in these experiments are shown in Table I.

TABLE I

ANALYSIS OF KNOWN EPICHLORHYDRIN ATMOSPHERES

Concentration of epichlorhydrin in atmosphere, mg per cubic metre	Volume of air sampled, litres	Epichlorhydrin found in first absorber, μg	Epichlorhydrin found in second absorber, μg	Epichlorhydrin in first absorber, %	Calculated concentration, mg per cubic metre
7·15	8·0	41	15	73·2	7·0
14·3	6·0	61	21	74·3	13·7
23·5	4·0	76	19	80·0	23·8
49·3	2·0	86	10	89·6	48·0
100·0	1·0	87	7	92·5	94·0

DISCUSSION OF RESULTS

The results obtained by the method on known test atmospheres indicate that the percentage of the epichlorhydrin trapped in the first bubbler decreases as the atmospheric concentration decreases. At 20 mg per cubic metre the efficiency of one bubbler is about 80 per cent., and this may be considered adequate for industrial hygiene investigations. For greater accuracy two bubblers should be used, or the appropriate correction factor for one bubbler applied.

The reason for the incomplete conversion of epichlorhydrin to formaldehyde is obscure, but may arise from an acid-catalysed polymerisation of epichlorhydrin with loss of the formaldehydogenic group. The results obtained by the method described are repeatable and there is little need for the initial heating in aqueous solution to convert epichlorhydrin to the corresponding glycol before oxidation. If the contents of the bubbler are set aside for a considerable period before analysis, there will be a gradual conversion to the glycol and somewhat higher results will be obtained, but as the over-estimate will not exceed

about 25 per cent. it is not likely to be a matter of concern in industrial hygiene investigations. If it is desired to avoid this source of error, the solution may be heated in a boiling-water bath for 1 hour to complete the conversion to the glycol before periodic acid oxidation; the final colour must be referred to a calibration graph prepared from epichlorhydrin solutions similarly treated.

The reaction used for the determination of epichlorhydrin in the atmosphere will also give positive results in the presence of formaldehyde or of substances that yield formaldehyde under conditions of the test. Compounds that contain, or that may be degraded to give, terminal vicinal hydroxy groups, such as ethylene oxide or ethylene glycol, will interfere.

The method is capable of giving an adequately accurate result with 20 μg of epichlorhydrin and is therefore capable of analysing an atmosphere containing 10 mg per cubic metre by means of a 2-litre sample. If lower concentrations are to be measured, a larger air sample should be taken and it would be desirable to use two bubblers. The reagent blank should be very low, but for the most accurate work it must be taken into consideration.

Technical assistance in this investigation was provided by Mr. Z. S. Berczy.

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Modifications of the Aniline Acetate - Furfural Method for the Determination of Pentose

By A. Borrow and E. G. Jefferys

The aniline concentration and reaction temperature of the method of Reeves and Munro have been modified, with an increase in sensitivity.

The method of Reeves and Munro[1] involves the conversion of pentose sugar to furfural by hydrochloric acid in the presence of a xylene phase, which removes the furfural as it is formed, the furfural then being measured colorimetrically at 540 mμ after treatment with aniline acetate. Davidson and Waymouth[2] devised apparatus to facilitate convenient handling of small samples. In these methods the colour was developed with a 1 per cent. v/v solution of aniline in a mixture of ethanol and glacial acetic acid (1 + 1 v/v), in the dark at room temperature. It has been shown that raising the aniline concentration and lowering the temperature result in an increase in sensitivity.

Experimental

Effect of temperature and light—

A stock solution of furfural in xylene was prepared from xylose by the method described.[1] Five-millilitre samples were taken and the colour was developed over a range of temperatures in the dark or in daylight, the 1 per cent. v/v aniline reagent being used. Readings were taken rapidly to minimise interference from the colorimeter lamp. Separate tubes in individual light-proof containers were used for each measurement of the colour development in the dark, whereas the colour development in single tubes was followed in daylight. It will be seen in Fig. 1 that the colour developed to a maximum intensity for each condition and then began to decrease. The initial rate of colour development was rapid and appeared to be independent of these conditions. The results suggest that colour development and decay were occurring simultaneously. Therefore the maximum intensity depended upon the rate of decay, which was sensitive to both light and temperature. It was decided that, with the added sensitivity gained owing to working at low temperatures, the small gain in sensitivity resulting from working in the dark did not justify the experimental complications involved.

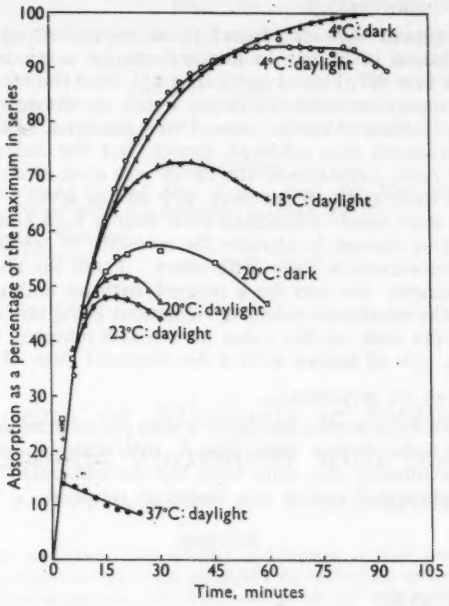

Fig. 1. Effect of temperature and light on colour development of the aniline - furfural reaction

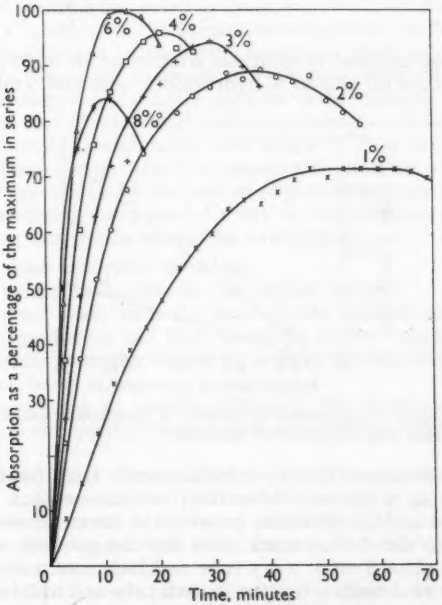

Fig. 2. Effect of aniline concentration on the colour development of the aniline - furfural reaction

EFFECT OF ANILINE CONCENTRATION—

The colour development was also found to be dependent upon the concentration of aniline. Six concentrations of aniline in ethanol - glacial acetic acid mixture (1 + 1 v/v) were employed, each at four dilutions of furfural at 4° C, and the mean absorptions, expressed as a percentage of the maximum achieved in the series, are shown in Fig. 2. It will be seen that with rising concentrations of aniline from 1 to 6 per cent. v/v the colour intensity was increased, and the maximum was achieved sooner, but the colour began to decay more rapidly. At the 8 per cent. aniline level the decay was even more rapid, so that the maximum reached was less than at the 6 per cent. v/v aniline level.

It is desirable to work under conditions such that a high colour intensity is achieved rapidly, but enduring long enough to obviate the necessity of precise timing, and that small variations in aniline concentration have little effect. From the results in Fig. 2, it will be seen that these requirements are met by a reagent with an aniline concentration between 4 and 6 per cent. v/v, the maximum colour development being reached in 21 and 14 minutes and enduring within 2 per cent. of this value for 9 and 8 minutes, respectively. A concentration of 4·5 per cent. v/v of aniline with a development time of 18 minutes was chosen.

CONVERSION OF PENTOSE TO FURFURAL—

The production of furfural from a 400 μg of xylose per ml standard was shown to increase with time of heating under reflux with 5·55 *N* hydrochloric acid, but the increase in sensitivity gained by extending this time from the recommended 2½ to 4 hours was only 5 per cent. The recommended period was therefore retained.

METHOD

REAGENTS—

Hydrochloric acid, 5·55 N.

Xylene, redistilled.

Sodium acetate, anhydrous—Analytical-reagent grade.

Aniline reagent—A 4·5 per cent. v/v solution of analytical-reagent grade aniline in ethanol - glacial acetic acid mixture (1 + 1 v/v).

PROCEDURE—

By pipette put 1·0-ml aliquots of solutions containing 10 to 80 μg of pentose into tubes calibrated at 7·0 ml; add 2·0 ml of 5·55 *N* hydrochloric acid and 5 ml of xylene, fit the tubes

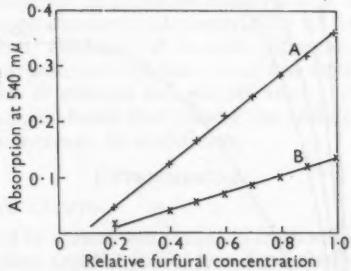

Fig. 3. Comparison of results: A, proposed method; B, Davidson and Waymouth's method

with air condensers and immerse them in a boiling-water bath for 2½ hours. Also include a standard containing 50 μg of xylose. After the tubes have cooled, remove the condensers, put stoppers in the tubes and shake them; remove the lower aqueous layer through a fine capillary. Add xylene to the 7·0-ml mark, and dry the solution with anhydrous sodium acetate. With a pipette place 5 ml in a dry tube and then cool it in an ice-bath. Add 5 ml of freshly prepared and cooled aniline reagent to each tube and also to the 5-ml xylene blank. Measure the colour at 540 mμ on a Unicam SP350 spectrophotometer, after development for 18 minutes in an ice - water bath.

Results

The colour development of the modified procedure was compared with that of Davidson and Waymouth,[2] dilutions of a standard solution of furfural in xylene being used. The results are shown in Fig. 3 (1·0 ≡ 75 μg of xylose per ml). The whole method gave a linear response over the range 10 to 80 μg of xylose, with a maximum deviation from the mean of 4·7 per cent. on xylose standards measured on separate occasions.

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The Determination of Alkaloids in Biological Material by Compound Formation with Indicators

By Z. I. El Darawy and S. L. Tompsett

The use of indicators and related substances in the determination of basic organic compounds such as alkaloids in biological materials has been investigated. Concentration has been achieved by adsorption on Florisil and specificity by paper chromatography.

One of the most sensitive methods for the quantitative determination of nitrogenous bases is that in which use is made of compound formation with indicators in organic solvents. A survey of this type of method has recently been published by Ballard, Isaacs and Scott,[1] who quote references. The object of the present investigation was to apply such a procedure to the determination of alkaloids in biological materials such as urine, blood plasma and soft tissues. For such a procedure to be of any practical value, it was found necessary to introduce certain initial stages. Materials such as urine contain, as normal constituents, substances that interfere, and it was found necessary to reduce their effect. Low concentrations of alkaloid are usually encountered and hence it was necessary to incorporate a concentration stage. The reaction itself is non-specific and so an attempt was made to achieve some degree of specificity by the use of paper chromatography.

For the reaction involving compound formation with indicators in organic solvents to be reliable, the following conditions should be satisfied—

(*a*) the indicator must be acidic in nature,
(*b*) the indicator should be soluble in the organic solvent,
(*c*) the indicator must react with the base (in the organic solvent) in stoicheiometric proportions to produce a salt that is soluble in the organic solvent,
(*d*) the base - indicator complex should be readily decomposed by aqueous solutions of strong acids or bases,
(*e*) the organic base must be readily soluble in the organic solvent, and
(*f*) the organic solvent should have a low solubility in water and a relatively high boiling point.

Although the bromothymol blue - benzene technique was finally used, a considerable amount of preliminary work was carried out in an examination of various indicator - solvent systems and their reaction towards a number of alkaloids. A summary of this work is given in what follows; the results suggest that some degree of specificity can be achieved by the use of various indicator - solvent systems.

The alkaloids and allied bases examined were strychnine, brucine, atropine, hyoscine, hyoscyamine, cocaine, procaine, heroin, quinine, lobeline, aconitine, benzocaine, yohimbine and morphine. Morphine was found to be non-reactive towards any system examined, this

being due to the phenolic group. The following indicator - solvent systems were found to be reactive towards certain of the above-named compounds, the value in brackets being the pH of the buffered solution as indicator—

(*i*) Solvent: benzene.
Indicators: methyl orange (pH 5·0), metanil yellow (pH 4·6), bromothymol blue (pH 7·4), bromocresol purple (pH 6·5), bromophenol blue (pH 5·0), bromocresol green (pH 5·5).
All compounds reacted except benzocaine and yohimbine.

(*ii*) Solvent: ethylene dichloride.
Indicators: methyl orange (pH 5·0), metanil yellow (pH 4·6), bromocresol purple (pH 6·5).
All compounds reacted.

(*iii*) Solvent: ether.
A. Indicators: methyl orange (pH 5·0), metanil yellow (pH 4·6).
No compound reacted.
B. Indicators: bromocresol green (pH 5·5), bromocresol purple (pH 6·5), bromophenol blue (pH 5·0).
Only strychnine, brucine, quinine, lobeline and yohimbine reacted.

The systems examined quantitatively with quinine as the test alkaloid were: methyl orange - benzene; bromothymol blue - benzene; methyl orange - ethylene dichloride; metanil yellow - ethylene dichloride. Beer's law was followed within the range 0 to 20 μg of alkaloid.

Experimental examinations were almost entirely confined to strychnine and the bromothymol blue - benzene system. The scope of this work fell into two parts (*a*) the determination of quantities such that a preliminary concentration procedure was unnecessary, and (*b*) the determination of quantities such that a preliminary concentration procedure was required.

Determination without preliminary concentration

Determination in urine—

Normal urine was examined by means of a number of the indicator - solvent systems. There was no history of administration of alkaloids or related compounds to the subjects whose urine was used. Positive results were obtained in a number of systems, particularly the very sensitive bromothymol blue - benzene, the extracted indicator being equivalent to about 3 μg of alkaloid (as strychnine) per ml of urine. The nature of the bases in normal urine that so react is unknown. Some workers, *e.g.*, Oberst,[2] have suggested the use of an empirical blank, based on an average value obtained from a large number of normal urines. We consider that such a device introduces too large an error, particularly in the determination of an alkaloid in low concentrations.

It has been usual to add 1 ml of 2 *N* sodium hydroxide to 10 ml of aqueous alkaloid solution before extraction with an organic solvent. The pH of such a mixture exceeds 12. It has been found however that, when the bromothymol blue - benzene system is used, if the pH of the initial aqueous phase is adjusted to a value between 8 and 9 only, then the blank obtained from "normal" urine is negligible, yet the recovery of added strychnine is quantitative. The following procedure has been found satisfactory.

Procedure—Adjust the pH of 10 ml of urine to between 8 and 8·5 by adding 0·1 *N* sodium hydroxide; add 25 ml of benzene and shake the mixture for 10 minutes. Then spin it in a centrifuge, and shake 20 ml of the separated benzene layer with 0·5 ml of buffered (0·1 per cent. in *M*/15 phosphate buffer, pH 7·4) bromothymol blue solution for 5 minutes. Spin the mixture in a centrifuge, then remove 15 ml of the benzene layer and shake with 4 ml of aqueous 0·1 *N* sodium hydroxide solution. Take readings for the coloured aqueous solution against a blank in a spectrophotometer at 510 mμ. In 8 experiments the recovery of 50 μg of strychnine added to 10 ml of urine ranged from 89 to 102 per cent. (44·4 to 51·1 μg).

Determination in plasma—

Initially the bromothymol blue - benzene procedure was applied to plasma made strongly alkaline by the addition of sodium hydroxide, the pH exceeding 12. In contrast to that from urine the blank was very low, being about 0·1 to 0·2 μg (as strychnine) per ml of plasma.

Blank values of the same magnitude were obtained when protein-free extracts of plasma were used and prepared by the use of trichloroacetic or tungstic acids.

The procedure adopted is similar to that described for urine, 10 ml of plasma being used. Strychnine added to plasma in quantities ranging from 1·5 to 5·0 μg per ml could be recovered almost quantitatively by this procedure. Other results are shown in Table I.

TABLE I

RECOVERY OF STRYCHNINE ADDED TO 10 ml OF PLASMA

Strychnine added, μg per ml	Strychnine recovered, μg per ml	Recovery, %
5·0	4·95	99
	4·95	99
	4·80	96
	5·00	100
	5·05	101
3·0	2·80	93
	2·90	97
	2·45	82
	2·80	92
1·5	1·20	80
	1·40	93
	1·35	90

DETERMINATION IN TISSUES—

Investigations were carried out with liver and muscle. The following procedure has been found satisfactory.

Procedure—Put 75-g of tissue (liver or muscle) and 75 ml of 0·85 per cent. w/v sodium chloride solution in a high-speed macerator. For experimental purposes the alkaloid was added to the tissue brei and thoroughly mixed in by further maceration. Heat a 10-ml portion of this suspension and 10 ml of water in a boiling-water bath for 10 minutes. Add 1 ml of 5 *N* hydrochloric acid to the mixture, and continue the heating for a further 10 minutes. After cooling the mixture, spin it in a centrifuge and separate the supernatant fluid. Add sufficient 20 per cent. trichloroacetic acid in *N* hydrochloric acid to the supernatant fluid to precipitate any traces of protein. Spin the mixture in a centrifuge, and examine an aliquot of the supernatant fluid by the procedure described for urine.

TABLE II

RECOVERY OF STRYCHNINE ADDED TO LIVER BREI OR MINCED MUSCLE

Material	Strychnine taken, μg per g	Recovery, %
Liver	10	94·8, 106·8, 102·5
	20	96·4, 105·2, 100·0, 91·2
Muscle	10	100, 102
	20	101·5, 101·5, 100

It will be seen from the results shown in Table II that strychnine added to liver or muscle in quantities of 10 or 20 μg per g could be recovered quantitatively by this procedure.

Tissue extracts in contrast to urine resembled plasma in that the use of even strongly alkaline extracts produced very low blank values. These were about 0·4 μg (as strychnine) per g of wet tissue.

DETERMINATION WITH PRELIMINARY CONCENTRATION

In toxicological practice, minute quantities of alkaloids may be present in a large volume of urine or plasma, giving a concentration far lower than those at which the direct determination method is accurate. Concentration methods have therefore been studied. The following have been found to be unsatisfactory—

(*a*) simple evaporation,
(*b*) freezing and partial thawing,
(*c*) partial dehydration by the addition of anhydrous sodium sulphate, and
(*d*) extraction by immiscible solvent.

The most satisfactory general procedure has been found to consist of adsorption on Florisil* followed by elution with an aqueous ethanolic sodium carbonate solution. Optimum adsorption of strychnine on Florisil was found to be related to the pH of the solution, suitable ranges being pH 5 to 6 for aqueous solutions and urine, pH 8 to 9 for plasma and pH 7·0 to 8·5 for liver extracts.

REAGENTS—

Florisil, 60 to 100 mesh.

Alkaline eluting agent—Mix 75 parts of ethanol with 20 parts of water and 5 parts of 5 per cent. w/v sodium carbonate solution.

PREPARATION OF COLUMN—

Pack a column with Florisil (15 cm long; 1 cm in diameter). Place cotton-wool pads at the top and the bottom of the column to retain any solid particles. Wash with 100 ml of 10 per cent. ammonia solution (10 ml of ammonia solution, sp.gr. 0·880, per 100 ml), followed by 200 ml of water.

PROCEDURE FOR URINE—

Allow a convenient volume, *e.g.*, 250 ml, of urine (pH adjusted to between 5 and 6) to percolate through the column. Wash the column with 100 ml of 0·5 per cent. ammonia solution (5 ml of ammonia solution, sp.gr. 0·880, per litre), then with 100 ml of water and finally with 50 ml of 30 per cent. acetone. All urinary pigments are thereby removed, leaving the alkaloid intact on the column.

Add 6 ml of 5 per cent. w/v aqueous sodium carbonate solution to the column and then treat it with 75 ml of eluting agent. Acidify the eluate with 2 ml of 5 *N* hydrochloric acid and evaporate the mixture to dryness on a water bath. Dissolve the residue in 10 ml of 0·1 *N* hydrochloric acid, and examine an aliquot of this by the bromothymol blue - benzene procedure.

"Normal" urine when examined by this procedure was found to give negative results, whereas strychnine added to urine could be recovered almost quantitatively (Table III).

PROCEDURE FOR PLASMA—

Dilute 50-ml of plasma five times with water and adjust the pH to a value between 8 and 9·0. The procedure is then essentially the same as that described for urine.

A flocculent precipitate may be left after evaporation of the acidified eluate. This is due to adsorption of part of the plasma proteins on the Florisil and their subsequent extraction by the alkaline eluate.

Strychnine added to plasma could be determined satisfactorily by this procedure (Table III).

PROCEDURE FOR TISSUES—

Homogenise 50 g of liver with 100 ml of 0·85 per cent. aqueous sodium chloride solution. Place the mixture in a boiling-water bath for 5 minutes, add 50 ml of 2 *N* hydrochloric acid and heat the mixture for a further 10 minutes. After cooling the mixture, dilute it to 250 ml with water and filter it.

Examine an aliquot of the filtrate, with pH adjusted to between 7·0 and 8·5, as described for urine.

Strychnine added to liver could be recovered quantitatively by this procedure (Table III).

TABLE III

RECOVERY OF STRYCHNINE ADDED TO URINE, PLASMA AND TISSUES AFTER CONCENTRATION ON FLORISIL

Material	Amount taken	Strychnine taken	Recovery, %
Aqueous solution ..	100 ml	1·0 μg per ml	95·6, 97·0, 101·5
Urine	250 ml	0·5 μg per ml	95, 90, 92
Plasma	50 ml	2·0 μg per ml	80*, 94†, 92·5†
Liver	50 g	2·0 μg per g	92·5, 94·7, 96·0

* Absorption at pH 5 to 6. † Absorption at pH 8 to 9.

* A very convenient synthetic silicate obtainable in various standard particle sizes (we used 60 to 100 mesh, standard U.S. equivalent sieves). It is obtainable from the Floridin Co. Inc., Pa., U.S.A.

Use of paper chromatography to increase specificity

Although the indicator - solvent system represents a sensitive and accurate method for the determination of a single alkaloid in biological materials, it lacks specificity. The present section is concerned with an attempt to repair this deficiency. Strychnine and brucine, both of which form a benzene-soluble complex with bromothymol blue, were used as test alkaloids. They form a specially suitable test mixture, because in toxicological practice a mixture of the two alkaloids may be encountered and their separate determination may be an important factor in determining the source of toxic material. Attempts were made to separate the indicator - alkaloid complexes by adsorption or partition chromatography, but these were unsuccessful. A separation of the alkaloids themselves by paper chromatography and their subsequent elution and determination by the bromothymol blue - benzene technique, however, proved successful.

The complete procedure now resolves itself into the following stages—

(*a*) preparation of plasma or tissue extract,
(*b*) adsorption on Florisil,
(*c*) elution from Florisil,
(*d*) separation by paper chromatography, and
(*e*) elution and determination by the bromothymol blue - benzene technique.

It was necessary to effect the following changes in the technique previously described—

(*i*) The alkaloids are eluted from the Florisil column with ethanolic ammonia solution instead of ethanolic sodium carbonate solution. The latter leaves a large residue of inorganic salts upon evaporation, and this is undesirable for the paper-chromatographic part of the technique. The ammoniacal eluting agent leaves no appreciable residue upon evaporation. The trace of material left does, however, produce a positive reaction in the bromothymol blue - benzene reaction, owing to the formation of ethylamine, but this does not interfere in the present procedure, since ethylamine has an R_F value entirely different from those of the alkaloids under investigation.

(*ii*) Protein-free extracts of plasma were used rather than diluted plasma, since with the latter a fraction of the plasma proteins would be adsorbed on and eluted from the Florisil column. The presence of such material would be undesirable in the paper-chromatographic part of the technique.

Reagents—

Alkaline eluting agent—Mix 80 parts of ethanol with 15 parts of ammonia solution, sp.gr. 0·880, and 5 parts of water.

Sodium dihydrogen phosphate, 0·25M.

n-*Butanol, saturated with water*.

Dragendorff reagent—Solution A: dissolve 0·85 g of bismuth subnitrate in 40 ml of water and 10 ml of glacial acetic acid; solution B: dissolve 8 g of potassium iodide in 20 ml of water. Before use, add 5 ml of solution A, 5 ml of solution B and 20 ml of glacial acetic acid to 100 ml of water.

Apparatus—

Tank for ascending chromatography.

Whatman filter-paper No. 4, strips 8 cm × 60 cm.

Procedure for aqueous solutions—

Allow 200 ml of an aqueous solution of strychnine and brucine, pH value adjusted to 6, to percolate through a column of Florisil, and wash the column with 100 ml of water. When the water surface is only 0·5 cm from the top of the column, add 0·5 ml of ammonia solution, sp.gr. 0·880, to furnish a strongly alkaline medium for the elution of the alkaloids. Elute with 75 ml of the alkaline eluting agent. Evaporate to dryness and dissolve the residue in 1 ml of 96 per cent. ethanol.

Impregnate paper strips of Whatman No. 4 filter-paper, 80 mm × 600 mm, with 0·25*M* sodium dihydrogen phosphate solution. Apply 0·4 ml of the ethanolic solution of the residue along a line 4 cm from one edge of the paper strip, allowing the ethanol to dry after each addition. Expose the filter-paper strip to steam for 5 minutes and then hang it in the chromatographic tank for 2 to 6 hours to attain equilibrium with the atmosphere (*n*-butanol

and water vapours). Dip the end of the paper in the solvent (*n*-butanol saturated with water), and allow the solvent front to travel a distance of 35 to 40 cm up the paper (about 24 hours).

Remove the paper strip from the tank, dry it and spray it with the modified Dragendorff reagent to reveal the position of the alkaloids.

Cut out the areas shown to contain the alkaloid spots and place them in test-tubes containing 3 ml of *N* hydrochloric acid. Place the tubes in a boiling-water bath for 10 minutes and set them aside at room temperature for 2 hours. Squeeze the solution out of the paper with a glass rod, and wash the paper with a further 1 ml of water.

Determine the alkaloid content of the extract by the bromothymol blue - benzene technique.

PROCEDURE FOR URINE—

The procedure is that described above; use 250 ml of urine, with the pH adjusted to 6·0. Before elution of the alkaloids adsorbed on the Florisil column, wash the column with 100 ml of 0·5 per cent. ammonia solution, 100 ml of water, followed by 50 ml of 30 per cent. acetone to remove adsorbed pigments.

PROCEDURE FOR PLASMA—

Into a flask measure 75 ml of plasma, 45 ml of 26 per cent. trichloroacetic acid in *N* hydrochloric acid and water to a volume of 500 ml. Set the mixture aside for 1 hour and then filter it.

Adjust the pH of 400 ml of the filtrate to 6, and then proceed as described for aqueous solutions.

PROCEDURE FOR TISSUES (*e.g.* LIVER)—

Prepare a trichloroacetic acid extract of liver as described on p. 603 and, after the pH has been adjusted to 7·0 to 8·5 examine it as described for aqueous solutions.

TABLE IV

RECOVERY OF STRYCHNINE AND BRUCINE

Material	Amount taken	Alkaloid taken	Recovery, %
Aqueous solution ..	200 ml	100 μg of strychnine	92, 92, 89
		100 μg of brucine	81, 95, 94
Urine	250 ml	100 μg of strychnine	103, 97
		100 μg of brucine	96, 93
Plasma	75 ml	50 μg of strychnine	91, 97
		50 μg of brucine	93, 93
Liver	50 g	100 μg of strychnine	84, 93
		100 μg of brucine	82, 93

It will be seen from the results in Table IV that added strychnine and brucine could be recovered satisfactorily by the procedures described.

In conclusion we thank Dr. C. P. Stewart for his helpful criticism and advice during the course of this investigation.

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DEPARTMENT OF CLINICAL CHEMISTRY
UNIVERSITY OF EDINBURGH

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Recommended Methods for the Analysis of Trade Effluents

PREPARED BY THE JOINT A.B.C.M. - S.A.C. COMMITTEE ON METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS

Methods for the Determination of Chromium, Lead and Selenium

Chromium

TOTAL CHROMIUM

PRINCIPLE OF METHOD—

After destruction of the organic matter, all the chromium present is oxidised to chromate, which is determined colorimetrically as the violet-coloured complex with diphenylcarbazide.

RANGE—

For chromium contents up to 20 μg.

REAGENTS—

Distilled water—This should be specially prepared by distilling tap water to which sulphuric acid and a few crystals of potassium permanganate have been added. Suitable precautions must be taken to exclude atmospheric dust during distillation and storage.

This specially prepared water must be used for the reagents and throughout the procedure.

Sulphuric acid, sp.gr. 1·84.

Nitric acid, sp.gr. 1·42.

Ammonium oxalate solution, saturated.

Sodium sulphite—$Na_2SO_3.7H_2O$.

Phosphoric acid, 60 per cent.

Potassium permanganate solution, 1 per cent. w/v.

Sodium hydroxide solution, 15 per cent. w/v.

Hydrogen peroxide, 3 per cent. v/v (10-volume).

**Diphenylcarbazide solution*—Dissolve 0·25 g of diphenylcarbazide in 25 ml of ethanol and dilute to 100 ml with distilled water.

**Sulphuric acid, dilute, 5 per cent. v/v.*

**Standard chromium solution*—Dissolve 0·3740 g of potassium chromate in distilled water and dilute to 1 litre. Dilute 10 ml of this solution to 500 ml. This solution should be freshly prepared as required.

1 ml $\equiv$ 2 μg of chromium.

PROCEDURE—

Place 100 ml (or a suitable volume) of the effluent sample in a 250-ml Kjeldahl flask and dissolve in it 0·1 g of sodium sulphite. Add 2 ml of concentrated sulphuric acid and evaporate until white fumes of sulphur trioxide are evolved. If necessary, add concentrated nitric acid drop by drop to oxidise any residual organic matter. Add 10 ml of saturated ammonium oxalate solution and evaporate once more to fumes. When cool, dilute the solution with 10 ml of distilled water and transfer the contents of the Kjeldahl flask to a 25-ml calibrated flask, and dilute to the mark.

Mix well and place 5 ml, or a larger aliquot if necessary, in a small beaker, add 5 drops of phosphoric acid and evaporate to fumes. Cool the solution, add 1 ml of potassium permanganate solution, cover the beaker with a watch-glass and heat on a water bath for 20 minutes.

Neutralise the solution to litmus paper with sodium hydroxide solution and add 1 ml in excess. Add 2 ml of the hydrogen peroxide and allow the solution to simmer gently on a hot-plate for 10 minutes. Cool the solution, dilute it to 20 ml in a calibrated flask and filter. Measure accurately a volume of the filtrate (5 to 10 ml) into a 25-ml calibrated flask, add 5 ml of dilute sulphuric acid and dilute the solution to about 20 ml. Add 2·5 ml of diphenylcarbazide solution and dilute the solution to the mark. Allow the solution to stand for 5 minutes before the colour measurement.

Carry out a blank determination on all the reagents used.

Measure the optical density in a spectrophotometer or an absorptiometer, using a wavelength of 5400 A in a spectrophotometer, or a suitable green filter in an absorptiometer. Read the number of micrograms of chromium equivalent to the observed optical density of the test and blank solutions from a previously prepared calibration graph, and so obtain the net measure of chromium in the sample.

As the violet colour fades on standing, the colour measurement should be carried out after 5 minutes.

Establish the calibration graph as follows—

Measure appropriate amounts of standard chromium solution covering the range 0 to 20 μg of chromium into a series of 25-ml calibrated flasks. Add to each 5 ml of dilute sulphuric acid and dilute the solution to about 20 ml. Add 2·5 ml of diphenylcarbazide solution and dilute each solution to the mark. Allow the solutions to stand for 5 minutes and then measure the optical densities and construct a graph relating the optical densities to the number of micrograms of chromium.

If an instrument is not available, colours may be visually matched against a series of standards.

Chromium present in the effluent as chromate

It may sometimes be useful to determine the chromium present as chromate, and the method below is recommended, with the following reservations—

(*a*) it is not applicable when the effluent itself is highly coloured, and
(*b*) chromate tends to disappear in the effluent owing to reduction by organic impurities.

Principle of method—

The chromate in the effluent sample is directly determined colorimetrically as the violet-coloured complex with diphenylcarbazide.

Range—

For chromium contents up to 20 μg.

Reagents—

Those marked with an asterisk (*) in the method for "Total Chromium."

Procedure—

Place 25 ml of the effluent sample in a 50-ml cylinder and add 5 ml of diphenylcarbazide solution; then add 10 ml of dilute sulphuric acid, with mixing, and dilute the solution to 50 ml. Allow it to stand for 5 minutes and measure the optical density as described in the method for "Total Chromium."

If the solution under test is turbid, clear it before applying the test either by centrifuging it or by filtering it in an alkaline condition, using a filter aid.

Lead

Principle of method—

After destruction of the organic matter, lead is extracted from ammoniacal cyanide solution as the pink lead dithizonate, interfering metals being first removed by extraction in acid solution. The lead is then determined colorimetrically.

RANGE—

For lead contents of (*a*) up to 100 μg (instrumental method)
or (*b*) up to 15 μg (visual colour-comparison method).

APPLICABILITY—

The method is generally applicable. Relatively large amounts of calcium phosphate interfere.

APPARATUS—

NOTE—Lead-free resistance-glass apparatus must be used throughout.

Titration flask—This is shown in Fig. 1.

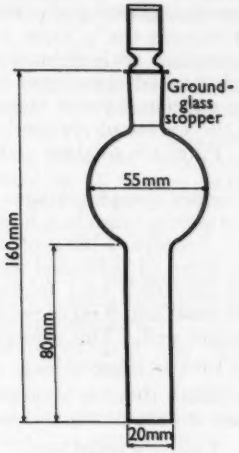

Fig. 1. Titration flask

REAGENTS—

NOTE—Lead-free distilled water must be used throughout.

Chloroform, redistilled—Redistil chloroform in an all-glass distillation apparatus.

Ammonium hydroxide, approximately 10 M—Dilute 1 volume of ammonium hydroxide, sp.gr. 0·880, with 1 volume of distilled water.

Ammonium hydroxide, diluted—Dilute 1 volume of ammonium hydroxide, sp. gr. 0·880, with 2 volumes of distilled water.

Hydrochloric acid, sp.gr. 1·18, redistilled.

Hydrochloric acid, diluted (1 + 1).

Nitric acid, redistilled—Redistil nitric acid, sp.gr. 1·42, in an all-glass distillation apparatus.

Nitric acid, diluted (1 + 3).

Nitric acid, dilute (*about* 1 *per cent.*).

Diphenylthiocarbazone (*dithizone*) *stock solution*—Dissolve 0·05 g of dithizone in 100 ml of redistilled chloroform.

Dithizone extraction solution A—Extract 15 ml of the dithizone stock solution with two 50-ml portions of dilute ammonium hydroxide (50 ml of distilled water containing 2 ml of 10 *M* ammonium hydroxide) and reject the chloroform layer each time. Filter the combined ammoniacal extracts if necessary. Acidify the extract with diluted hydrochloric acid, and extract the precipitated dithizone with 100 ml of chloroform. Wash the extract with two 10-ml portions of distilled water, and filter it through a dry filter-paper. Prepare this solution freshly each day.

Dithizone extraction solution B—Dilute 20 ml of dithizone extraction solution A to 100 ml with chloroform.

Hydroxylamine hydrochloride solution—Dissolve 25 g of hydroxylamine hydrochloride in about 60 ml of distilled water, add 0·2 ml of thymol blue indicator solution and make alkaline with 10 *M* ammonium hydroxide to the full blue colour of the

indicator. Cool and extract the solution with dithizone extraction solution A, using 5-ml portions, until the last extract remains green; then wash the aqueous solution free from the excess of dithizone by repeated extraction with 10-ml portions of chloroform. Warm the solution until the excess of chloroform has been removed, cool, filter and dilute to 250 ml with distilled water.

Sodium citrate solution—Dissolve 150 g of sodium citrate, $Na_3C_6H_5O_7.2H_2O$, in distilled water and dilute to 500 ml. Add 3 drops of ammonium hydroxide and extract the solution with dithizone extraction solution A in slight excess; then wash the aqueous solution until it is free from the excess of dithizone by repeated extraction with 10-ml portions of chloroform. Warm the solution until the excess of chloroform has been removed, cool and filter.

Potassium cyanide solution—Dissolve 50 g of potassium cyanide in the minimum amount of distilled water and transfer the solution to a separating funnel. Dilute it to 100 ml and extract the solution with dithizone extraction solution A, using 5-ml portions to each of which 5 ml of chloroform has been added, until the last extract remains green and the aqueous layer is tinged yellow. Wash the aqueous solution until it is free from the excess of dithizone by repeated extraction with 10-ml portions of chloroform. Filter the solution and dilute to 500 ml with distilled water.

Ammonium hydroxide - potassium cyanide solution—Mix 45 ml of 10 *M* ammonium hydroxide and 40 ml of the potassium cyanide solution and dilute to 200 ml.

Standard lead solution A—Dissolve 0·160 g of lead nitrate, dried at 100° C, in 50 ml of distilled water and 10 ml of redistilled nitric acid, and dilute to 100 ml in a calibrated flask. This is the stock lead solution. Measure 10·0 ml of this solution into a 1-litre calibrated flask, add 9 ml of redistilled nitric acid, dilute to the mark with distilled water and mix well. This solution should be freshly prepared.

$$1 \text{ ml} \equiv 10 \ \mu\text{g of lead.}$$

Standard lead solution B—Dilute 10 ml of standard lead solution A to 50 ml in a calibrated flask. This solution should be freshly prepared.

$$1 \text{ ml} \equiv 2 \ \mu\text{g of lead.}$$

Methyl orange indicator solution—A 0·04 per cent. aqueous solution.
Thymol blue indicator solution—A 0·025 per cent. aqueous solution.
m-*Cresol purple indicator solution*—A 0·05 per cent. aqueous solution.

PROCEDURE—

Measure 100 ml of the effluent sample (see Note) into a 400-ml beaker, add 2 drops of methyl orange indicator solution, just acidify with redistilled nitric acid and then add 10 ml of the acid in excess. Evaporate the solution to a volume of 10 to 15 ml and transfer it to a small glass dish, using the minimum amount of distilled water for rinsing. Evaporate the solution to dryness on a steam-bath. Wash down the sides of the dish with 5 ml of redistilled nitric acid, and again evaporate the solution to complete dryness on the steam-bath. Transfer the dish to an electrically heated muffle furnace controlled at 490° to 500° C and ignite for 15 minutes. Allow the dish and contents to cool, add 3 ml of redistilled nitric acid and 15 ml of distilled water, and heat for 5 minutes on the steam-bath. Filter the contents through a small Whatman No. 540 filter-paper and wash the residue with small amounts of hot diluted nitric acid (1 + 3); cool the filtrate.

Add 2 ml of hydroxylamine hydrochloride solution, 2 ml of sodium citrate solution and 2 drops of thymol blue indicator solution. Add 10 *M* ammonium hydroxide until the indicator colour changes to full blue; cool again. The solution should be quite clear at this point; if not, re-acidify it and increase the amount of sodium citrate solution. Add 0·5 ml of 10 *M* ammonium hydroxide in excess and 3 to 5 ml of potassium cyanide solution. Transfer the solution to a 100-ml pear-shaped separating funnel and adjust the volume to about 35 ml.

Extract the lead with dithizone extraction solution A, using 3 ml for each extraction. Shake the funnel vigorously for 30 seconds after each addition until all the lead is extracted; this is indicated by two consecutive extracts remaining green. Collect the chloroform extracts in a second separating funnel and reject the aqueous

layer. Wash the combined chloroform extracts with 50 ml of distilled water and run the chloroform layer into the first funnel; wash the distilled water with two 5-ml portions of chloroform and add these washings to the main chloroform extract of the lead dithizonate.

Shake the chloroform extract with 25 ml of dilute nitric acid (1 per cent.) for 1 minute, allow the layers to separate and reject all but 0·5 ml of the chloroform layer; this 0·5 ml is left for the detection of the presence or absence of bismuth, which would interfere unless removed at this stage. Adjust the nitric acid extract to pH 2 by adding 2 or 3 drops of *m*-cresol purple indicator solution followed by the addition, drop by drop, of diluted ammonium hydroxide (1 + 2) until the colour of the indicator changes to orange-pink. Shake the contents of the separating funnel vigorously for 30 seconds; if bismuth is present the 0·5 ml of dithizone in chloroform will change colour.

If bismuth is present, remove it from the acid solution by repeated extractions with 5-ml portions of dithizone extraction solution A. When all the bismuth has been extracted, wash the solution free from dithizone with chloroform.

If bismuth is absent, reject the 0·5 ml of dithizone in chloroform solution and wash the acid solution free from dithizone with chloroform.

Carry out a blank procedure on all the reagents used.

Proceed to determine the lead colorimetrically, either by the instrumental method or by the visual colour-comparison method described below.

Instrumental method—Treat the blank and test solutions similarly. To the dithizone-free solution add 3 ml of the ammonium hydroxide - potassium cyanide solution and re-extract the lead by shaking with 3-ml portions of dithizone extraction solution A. Collect the chloroform extracts.

To the combined chloroform extracts add 10 ml of a diluted ammonium hydroxide - potassium cyanide solution (4 ml of 10 *M* ammonium hydroxide and 1 ml of potassium cyanide solution in 100 ml of distilled water), shake the mixture for 10 seconds, allow the layers to separate and return the chloroform layer to the first separating funnel, rejecting the aqueous layer. Again wash the chloroform solution with 10 ml of the diluted ammonium hydroxide - potassium cyanide solution, shake for 10 seconds and allow the layers to separate: repeat this washing of the chloroform layer once more. Filter the chloroform layer through a dry Whatman No. 41 filter-paper into a dry 25-ml calibrated flask, and wash out the separating funnel with successive small amounts of chloroform, filtering each washing into the flask; dilute the solution to the mark with chloroform and mix well.

Measure the optical densities of the test and blank solutions in a spectrophotometer or in an absorptiometer, using a 4-cm or a 1-cm cell according to the depth of colour, and using a wavelength of 5500 A in a spectrophotometer or a suitable yellow-green filter in an absorptiometer. Use chloroform in the comparison cell. Read the number of micrograms of lead equivalent to the observed optical densities of the test and blank solutions from a previously prepared calibration graph, and so obtain the net measure of lead in the sample.

Prepare the calibration graph as follows—

> Measure the appropriate amounts of standard lead solution A into a series of 100-ml pear-shaped separating funnels. For the 4-cm cell the standards should cover the range 0 to 25 μg of lead; for the 1-cm cell they should cover the range 0 to 100 μg of lead. To each add 10 ml of distilled water followed by 2 ml of redistilled nitric acid, 2 ml of hydroxylamine hydrochloride solution, 2 ml of sodium citrate solution and 2 drops of thymol blue indicator solution, mixing the solution well; then add 10 *M* ammonium hydroxide until the colour of the indicator changes to full blue, and add 1 ml in excess. Add 1 ml of potassium cyanide solution, mix and dilute the solution to 35 ml. Extract the lead, carrying out the whole operation as described in the Procedure, beginning at "Extract the lead with dithizone extraction solution A . . ." in the third paragraph. Measure the optical densities, using chloroform in the comparison cell. Correct each reading for the blank and construct a graph relating the optical densities to the number of micrograms of lead.

indicator. Cool and extract the solution with dithizone extraction solution A, using 5-ml portions, until the last extract remains green; then wash the aqueous solution free from the excess of dithizone by repeated extraction with 10-ml portions of chloroform. Warm the solution until the excess of chloroform has been removed, cool, filter and dilute to 250 ml with distilled water.

Sodium citrate solution—Dissolve 150 g of sodium citrate, $Na_3C_6H_5O_7.2H_2O$, in distilled water and dilute to 500 ml. Add 3 drops of ammonium hydroxide and extract the solution with dithizone extraction solution A in slight excess; then wash the aqueous solution until it is free from the excess of dithizone by repeated extraction with 10-ml portions of chloroform. Warm the solution until the excess of chloroform has been removed, cool and filter.

Potassium cyanide solution—Dissolve 50 g of potassium cyanide in the minimum amount of distilled water and transfer the solution to a separating funnel. Dilute it to 100 ml and extract the solution with dithizone extraction solution A, using 5-ml portions to each of which 5 ml of chloroform has been added, until the last extract remains green and the aqueous layer is tinged yellow. Wash the aqueous solution until it is free from the excess of dithizone by repeated extraction with 10-ml portions of chloroform. Filter the solution and dilute to 500 ml with distilled water.

Ammonium hydroxide - potassium cyanide solution—Mix 45 ml of 10 *M* ammonium hydroxide and 40 ml of the potassium cyanide solution and dilute to 200 ml.

Standard lead solution A—Dissolve 0·160 g of lead nitrate, dried at 100° C, in 50 ml of distilled water and 10 ml of redistilled nitric acid, and dilute to 100 ml in a calibrated flask. This is the stock lead solution. Measure 10·0 ml of this solution into a 1-litre calibrated flask, add 9 ml of redistilled nitric acid, dilute to the mark with distilled water and mix well. This solution should be freshly prepared.

$$1 \text{ ml} \equiv 10 \ \mu\text{g of lead.}$$

Standard lead solution B—Dilute 10 ml of standard lead solution A to 50 ml in a calibrated flask. This solution should be freshly prepared.

$$1 \text{ ml} \equiv 2 \ \mu\text{g of lead.}$$

Methyl orange indicator solution—A 0·04 per cent. aqueous solution.
Thymol blue indicator solution—A 0·025 per cent. aqueous solution.
m-*Cresol purple indicator solution*—A 0·05 per cent. aqueous solution.

PROCEDURE—

Measure 100 ml of the effluent sample (see Note) into a 400-ml beaker, add 2 drops of methyl orange indicator solution, just acidify with redistilled nitric acid and then add 10 ml of the acid in excess. Evaporate the solution to a volume of 10 to 15 ml and transfer it to a small glass dish, using the minimum amount of distilled water for rinsing. Evaporate the solution to dryness on a steam-bath. Wash down the sides of the dish with 5 ml of redistilled nitric acid, and again evaporate the solution to complete dryness on the steam-bath. Transfer the dish to an electrically heated muffle furnace controlled at 490° to 500° C and ignite for 15 minutes. Allow the dish and contents to cool, add 3 ml of redistilled nitric acid and 15 ml of distilled water, and heat for 5 minutes on the steam-bath. Filter the contents through a small Whatman No. 540 filter-paper and wash the residue with small amounts of hot diluted nitric acid (1 + 3); cool the filtrate.

Add 2 ml of hydroxylamine hydrochloride solution, 2 ml of sodium citrate solution and 2 drops of thymol blue indicator solution. Add 10 *M* ammonium hydroxide until the indicator colour changes to full blue; cool again. The solution should be quite clear at this point; if not, re-acidify it and increase the amount of sodium citrate solution. Add 0·5 ml of 10 *M* ammonium hydroxide in excess and 3 to 5 ml of potassium cyanide solution. Transfer the solution to a 100-ml pear-shaped separating funnel and adjust the volume to about 35 ml.

Extract the lead with dithizone extraction solution A, using 3 ml for each extraction. Shake the funnel vigorously for 30 seconds after each addition until all the lead is extracted; this is indicated by two consecutive extracts remaining green. Collect the chloroform extracts in a second separating funnel and reject the aqueous

layer. Wash the combined chloroform extracts with 50 ml of distilled water and run the chloroform layer into the first funnel; wash the distilled water with two 5-ml portions of chloroform and add these washings to the main chloroform extract of the lead dithizonate.

Shake the chloroform extract with 25 ml of dilute nitric acid (1 per cent.) for 1 minute, allow the layers to separate and reject all but 0·5 ml of the chloroform layer; this 0·5 ml is left for the detection of the presence or absence of bismuth, which would interfere unless removed at this stage. Adjust the nitric acid extract to pH 2 by adding 2 or 3 drops of *m*-cresol purple indicator solution followed by the addition, drop by drop, of diluted ammonium hydroxide (1 + 2) until the colour of the indicator changes to orange-pink. Shake the contents of the separating funnel vigorously for 30 seconds; if bismuth is present the 0·5 ml of dithizone in chloroform will change colour.

If bismuth is present, remove it from the acid solution by repeated extractions with 5-ml portions of dithizone extraction solution A. When all the bismuth has been extracted, wash the solution free from dithizone with chloroform.

If bismuth is absent, reject the 0·5 ml of dithizone in chloroform solution and wash the acid solution free from dithizone with chloroform.

Carry out a blank procedure on all the reagents used.

Proceed to determine the lead colorimetrically, either by the instrumental method or by the visual colour-comparison method described below.

Instrumental method—Treat the blank and test solutions similarly. To the dithizone-free solution add 3 ml of the ammonium hydroxide - potassium cyanide solution and re-extract the lead by shaking with 3-ml portions of dithizone extraction solution A. Collect the chloroform extracts.

To the combined chloroform extracts add 10 ml of a diluted ammonium hydroxide - potassium cyanide solution (4 ml of 10 *M* ammonium hydroxide and 1 ml of potassium cyanide solution in 100 ml of distilled water), shake the mixture for 10 seconds, allow the layers to separate and return the chloroform layer to the first separating funnel, rejecting the aqueous layer. Again wash the chloroform solution with 10 ml of the diluted ammonium hydroxide - potassium cyanide solution, shake for 10 seconds and allow the layers to separate: repeat this washing of the chloroform layer once more. Filter the chloroform layer through a dry Whatman No. 41 filter-paper into a dry 25-ml calibrated flask, and wash out the separating funnel with successive small amounts of chloroform, filtering each washing into the flask; dilute the solution to the mark with chloroform and mix well.

Measure the optical densities of the test and blank solutions in a spectrophotometer or in an absorptiometer, using a 4-cm or a 1-cm cell according to the depth of colour, and using a wavelength of 5500 A in a spectrophotometer or a suitable yellow-green filter in an absorptiometer. Use chloroform in the comparison cell. Read the number of micrograms of lead equivalent to the observed optical densities of the test and blank solutions from a previously prepared calibration graph, and so obtain the net measure of lead in the sample.

Prepare the calibration graph as follows—

Measure the appropriate amounts of standard lead solution A into a series of 100-ml pear-shaped separating funnels. For the 4-cm cell the standards should cover the range 0 to 25 μg of lead; for the 1-cm cell they should cover the range 0 to 100 μg of lead. To each add 10 ml of distilled water followed by 2 ml of redistilled nitric acid, 2 ml of hydroxylamine hydrochloride solution, 2 ml of sodium citrate solution and 2 drops of thymol blue indicator solution, mixing the solution well; then add 10 *M* ammonium hydroxide until the colour of the indicator changes to full blue, and add 1 ml in excess. Add 1 ml of potassium cyanide solution, mix and dilute the solution to 35 ml. Extract the lead, carrying out the whole operation as described in the Procedure, beginning at "Extract the lead with dithizone extraction solution A . . ." in the third paragraph. Measure the optical densities, using chloroform in the comparison cell. Correct each reading for the blank and construct a graph relating the optical densities to the number of micrograms of lead.

Visual colour-comparison method—Treat the blank and test solutions similarly. Into the special titration flask (see Fig. 1) measure an aliquot of the dithizone-free solution, to contain 10 to 15 μg of lead. Dilute the solution to 25 ml with nitric acid (1 per cent.) and adjust the solution to pH 2 by adding 2 or 3 drops of *m*-cresol purple indicator solution, followed by the addition, drop by drop, of diluted ammonium hydroxide (1 + 2) until the colour of the indicator changes to orange-pink. Add 3 ml of the ammonium hydroxide - potassium cyanide solution, followed by 10 ml of dithizone solution B, and shake for 1 minute.

Prepare a comparison standard as follows—

To 5 ml of standard lead solution B in a similar flask, add 20 ml of dilute nitric acid (1 per cent.). Adjust the solution to pH 2 by the addition of 2 or 3 drops of *m*-cresol purple indicator solution, followed by the addition, drop by drop, of diluted ammonium hydroxide (1 + 2) until the colour of the indicator changes to orange-pink. Add 3 ml of the ammonium hydroxide - potassium cyanide solution, followed by 10 ml of dithizone solution B, and shake for 1 minute.

Compare the colour of the chloroform layer of the test with that of the standard, and then add standard lead solution B to the solution having the lower lead content (*i.e.*, the greener solution) until, after the solutions have been shaken and the layers allowed to separate, the colours of the two chloroform layers match. Add or subtract, as required, this added volume to or from 5·0 ml to obtain the volume of lead solution B equivalent to the lead present in the aliquot portion of the test solution taken. Correct for any lead found in the blank.

NOTE—Alternatively, the organic matter can be destroyed as described under "Destruction of Organic Matter"; then proceed to determine the lead in a suitable aliquot of the acid solution, beginning at "Add 2 ml of hydroxylamine hydrochloride solution . . ." in the second paragraph of the Procedure.

Selenium

Two methods are given—the first is a "sorting" test to establish the presence of selenium in a trade waste and to give an approximation of its content; the second is the recommended method of determination.[1] Visual colour measurement is recommended in both methods. Instrumental measuring is not recommended; the solution is colloidal, and although the colour can be matched either visually or nephelometrically, visual matching is to be preferred.

TEST TO ESTABLISH THE PRESENCE OF SELENIUM AND TO MEASURE THE APPROXIMATE CONTENT

PRINCIPLE OF METHOD—

After destruction of the organic matter, the selenium present in the effluent is reduced by means of either hydrazine sulphate or ascorbic acid to the elemental form, which is measured colorimetrically against standards.

RANGE—

For selenium contents up to 1·0 mg.

APPLICABILITY—

The method is generally applicable.

REAGENTS—

Hydrochloric acid, diluted (1 + 1),
Hydrazine sulphate,
or
Ascorbic acid.

Standard selenium solution—Dissolve 1 g of selenium in nitric acid, sp.gr. 1·42, and dilute the solution to 100 ml with distilled water. Measure a 10-ml aliquot into a beaker and add 5 ml of sulphuric acid, sp.gr. 1·84. Evaporate the solution until copious white fumes of sulphur trioxide are evolved. Cool and then add

5 ml of distilled water and repeat the evaporation. Cool the liquid and dilute with distilled water to 500 ml in a calibrated flask.

1 ml ≡ 0·2 mg of selenium.

PROCEDURE—

Destroy the organic matter in a volume of the effluent sample containing from 1 to 10 mg of selenium by wet oxidation, as described under "Destruction of Organic Matter," but omitting the addition of ammonium oxalate used in removing the excess of nitric acid. Dilute the filtrate to 100 ml with distilled water. Measure an aliquot not exceeding 10 ml into a large boiling-tube; if a smaller volume than 10 ml is taken, dilute it to 10 ml with distilled water in the tube. Add 30 ml of the diluted hydrochloric acid and heat the tube in a water bath maintained at 40° to 45° C until the temperature of the test liquid reaches 40° C; then add either (*a*) 0·25 g of hydrazine sulphate, or (*b*) 0·4 g of solid ascorbic acid, and mix. Maintain the temperature of the liquid at 40° to 45° C for 30 minutes. Simultaneously measure 1, 2, 3, 4 and 5 ml of the standard selenium solution into a series of boiling-tubes, dilute each to 10 ml, add 30 ml of hydrochloric acid and either hydrazine or ascorbic acid as before. Maintain the tubes containing the standards at the same temperature as the tube containing the test liquid for 30 minutes. Cool the solutions, transfer them to Nessler cylinders and dilute to 50 ml with distilled water. Match the sample with the standards.

RECOMMENDED METHOD OF DETERMINATION

PRINCIPLE OF METHOD—

After destruction of the organic matter by wet oxidation, the selenium in the effluent is distilled as the bromide, which in the aqueous distillate becomes selenous acid. This is reduced to elemental selenium by means of ascorbic acid and measured colorimetrically against standards.

RANGE—

For selenium contents up to 0·25 mg.

APPLICABILITY—

The method is generally applicable.

REAGENTS—

Nitric acid, sp.gr. 1·42.

Sulphuric acid, sp.gr. 1·84.

Perchloric acid, 60 *per cent.*

Sodium hydroxide solution, 1·0 N.

Hydrobromic acid, redistilled—Purify hydrobromic acid by distillation. Collect the colourless middle fraction of the distillate. Determine the hydrogen bromide content of the collected fraction by titrating 5 ml with *N* sodium hydroxide solution, using methyl orange as indicator.

Hydrobromic acid - bromine reagent—Mix 3 ml of bromine with 197 ml of hydrobromic acid.

Standard selenium solution A—Weigh 1·405 g of selenium dioxide and dissolve it in distilled water. Transfer the solution to a 1-litre calibrated flask, add 80 ml of hydrobromic acid and dilute the solution to the mark; mix well.

Standard selenium solution B—Measure 10 ml of selenium solution A into a 100-ml calibrated flask, add 1 ml of hydrobromic acid and dilute the solution to the mark; mix well. This solution should be freshly prepared as required.

1 ml ≡ 0·1 mg of selenium.

Ascorbic acid.

Methyl orange indicator solution—A 0·04 per cent. solution in 20 per cent. ethanol.

PROCEDURE—

Measure a suitable volume of the effluent sample into a beaker. Acidify the solution by the addition of nitric acid and add 5 ml in excess; then add 1·0 ml of perchloric acid. Evaporate the solution to about 10 ml and then cool it. Add

5 ml of sulphuric acid and heat the solution on a sand-bath until white fumes appear. If all the organic matter is not completely destroyed at this stage, cautiously add a further 1 ml of nitric acid and heat the solution again until white fumes appear. Allow the solution to cool; then add 10 ml of distilled water and heat the solution until white fumes appear. Repeat the treatment with distilled water and the evaporation a second time.

Cool the solution and transfer it to a 300-ml distillation flask, using 25 ml of distilled water for the transfer of the solution to the flask. Add 50 ml of hydrobromic acid and 6 ml of the hydrobromic acid - bromine reagent. Then slowly and carefully add 25 ml of sulphuric acid to the contents of the flask, cooling the flask during the addition. Place a boiling-rod inside the distillation flask. Fit an adaptor and water-cooled condenser to the flask and place a 100-ml conical flask, marked at 80 ml and containing 4 ml of hydrobromic acid - bromine reagent, at the condenser outlet; tilt the conical flask so that the tip of the condenser lies below the level of the reagent.

Heat the contents of the distillation flask, gently at first until the solution is boiling and afterwards more strongly, and allow the distillation to proceed until the volume of solution in the receiver is 80 ml. Remove the receiver and discontinue the distillation. Transfer the distillate to a 100-ml calibrated flask, dilute the solution to the mark with distilled water and mix.

PREPARATION OF STANDARD SELENIUM DISTILLATE—

Measure into a distillation flask similar to that used for the sample solution 25 ml of standard selenium solution B, and follow the same procedure for the distillation of selenium as described above, beginning with the addition of 50 ml of hydrobromic acid. Allow the distillation to proceed until the volume of solution in the receiver is 80 ml. Transfer the distillate to a 100-ml calibrated flask and dilute the solution to the mark with distilled water and mix well.

COLORIMETRIC DETERMINATION—

Determine the acidity of the sample and standard distillate solutions on an aliquot of each solution as follows—

> Measure 5 ml of the solution, add 100 ml of distilled water and boil the solution until it is free from bromine, but do not reduce the volume below 50 ml. Cool the solution, add 2 drops of methyl orange indicator and titrate with 1·0 *N* sodium hydroxide solution.

Measure aliquots of the standard distillate solution into a series of 50-ml Nessler cylinders, covering the range 0 to 0·25 mg of selenium in steps of 0·025 mg. From the results of the titrations calculate the volume of hydrobromic acid that must be put into each cylinder to make the acid content of each standard the same as that of 50 ml of sample distillate solution. Add these volumes and then dilute each standard to 50 ml with distilled water.

Measure 50 ml of the sample distillate solution into a 50-ml Nessler cylinder. Precipitate the selenium in the test and standard solutions by adding 0·4 g of ascorbic acid to each solution; stir the solutions until the ascorbic acid has dissolved. Allow the solutions to stand for 30 minutes and then visually match the coloured turbidity in the sample solution against the coloured turbidities in the standard solutions.

NOTE—The free bromine content of the sample and standard solutions will be different, but experience has shown that the shade and intensity of the coloured turbidity is not affected by any variation in the free bromine content of the selenium solution that occurs under the conditions described.

REFERENCE

1. Fogg, D. N., and Wilkinson, N. T., *Analyst*, 1956, **81**, 525.

Notes

CONTAMINATION DURING THE MICRO-DETERMINATION OF MAGNESIUM WITH 8-HYDROXYQUINOLINE

WHILE we were testing and comparing the performance of various methods for the micro-determination of magnesium in biological materials, before a study of hypomagnesaemia in ruminants, we were at first unable to obtain satisfactory results with the 8-hydroxyquinoline method described by Davidson.[1] The standard curve for 0 to 200 μg of magnesium, obtained by measuring the absorbancy of the reagent at 358 mμ after liberation from the oxinate by 0·1 *N* hydrochloric acid, was not linear and over most of its course lay above the linear theoretical curve calculated from the absorbancy of standard solutions of oxine and the bis composition of the magnesium complex (Table I). The subsequent analysis of various aliquots of solutions of ashed sheeps' faeces and urine gave variable results.

TABLE I

EFFECT OF CONTAMINATION ON STANDARD CURVE FOR MAGNESIUM

Magnesium added, μg	Absorbancy at 358 mμ (1-cm cell) in 25 ml of solution		
	Theoretical	High contamination	Negligible contamination
20	0·112	0·113	0·110
40	0·223	0·242	0·223
60	0·335	0·357	0·333
80	0·446	0·475	0·442
100	0·558	0·578	0·553
120	0·669	0·685	0·668
140	0·781	0·780	0·780
160	0·892	0·899	0·884
180	1·004	—	0·977
200	1·115	1·072	1·071

On further investigation we found that oxinate precipitates obtained from a pure magnesium solution by this method were heavily contaminated by zinc, although they had been washed twice with the ethanolic ammonia solution recommended by Davidson for removing the oxinates of zinc and several other metals that also react with the reagent under these conditions. This contamination, which was not evident from the blank values, was traced to two main sources, the face powder used by the technician who was assisting in the work and the rubber bungs used to stopper the centrifuge tubes during the incubation stage according to Davidson's instructions. The possibility of contamination from rubber bungs has long been recognised in trace-element work, but the effects of face powder and other cosmetics may not have been generally realised. Magnesium silicate (talc) and magnesium carbonate as well as zinc oxide are commonly used as major ingredients in the manufacture of face powder, so that the zinc contamination we observed was in all probability an indication of contamination by magnesium as well.

Smaller contributions to the zinc contamination were made by galvanised wire baskets in which the tubes were dried and by the centrifuge, which was an International model with a brass rotor. Since oxine reacts with a wide range of metals under the conditions employed in the method and the washing procedure appears to have a limited efficiency in the presence of appreciable amounts of magnesium oxinate, strict precautions must be taken to minimise contamination from such sources.

When the centrifuge tubes were left uncovered during the incubation stage and contamination from other sources had been reduced to a minimum, we found that in order to achieve quantitative precipitation of magnesium it was necessary to reduce the temperature of incubation from 80° C to 60° to 70° C and to increase the time of incubation from ½ hour to 1½ hours and the volume of added ammonia solution from 2 to 3 ml. The standard curve determined under these conditions followed the theoretical curve almost exactly for amounts of magnesium up to about 150 μg (Table I), whereas Davidson's curve deviates from linearity when the amount of magnesium present exceeds 100 μg. These deviations appear to be due to incomplete precipitation rather than to a failure to follow Beer's law.

Zinc was determined in oxinate precipitates obtained from 80, 120 and 200 μg of magnesium under these conditions by a dithizone method.[2] The unwashed precipitates contained 0·19, 0·24

and 0·14 μg of zinc, respectively, whereas those that had been washed twice with the ethanolic ammonia solution contained 0·64, 0·59 and 0·75 μg. The former values represent the zinc derived from the reagents, which were all of analytical-reagent grade, and the difference between the two levels is due to contamination from the centrifuge. However, since 1 μg of zinc causes a positive error equivalent to only 0·37 μg of magnesium, these amounts can be ignored. There were no significant amounts present of other metals that react with dithizone.

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1. Davidson, J., *Analyst*, 1952, **77**, 263.
2. Butler, E. J., D.Phil. Thesis, Oxford, 1952.

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THE DETERMINATION OF POTASSIUM BROMATE IN FLOUR

IT has been observed that iodine is liberated from potassium iodide in acidified "zinc filtrates" of flours that have been bleached by nitrogen peroxide. Consequently, determinations, by the author's method,[1] of bromate added to flours bleached with nitrogen peroxide yield high results. Depending upon a number of factors, the error may be slight or may be as great as or greater than 1·5 g per sack. Such errors can be eliminated completely by the addition, to the 50-ml aliquot of filtrate taken for analysis, of either—

(*a*) 5 ml of 0·5 per cent. w/v ammonium sulphamate after acidification of the filtrate, or
(*b*) 5 ml of a 7 per cent. w/v solution of hydroxylamine hydrochloride before acidification.

In either case the mixture should be set aside for 2 minutes before proceeding with the analysis.

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Errata

AUGUST (1956) ISSUE, p. 497, 4th line of text from foot of page (2nd line of "PROCEDURE"). For "at 1400 to 3000 **g***" read "at a relative centrifugal force of 1400 to 3000*".

IBID. The first 2 lines of the footnote *should read*—

"*The relative centrifugal force = $1{\cdot}12 \times 10^{-5} \times rN^2$,"